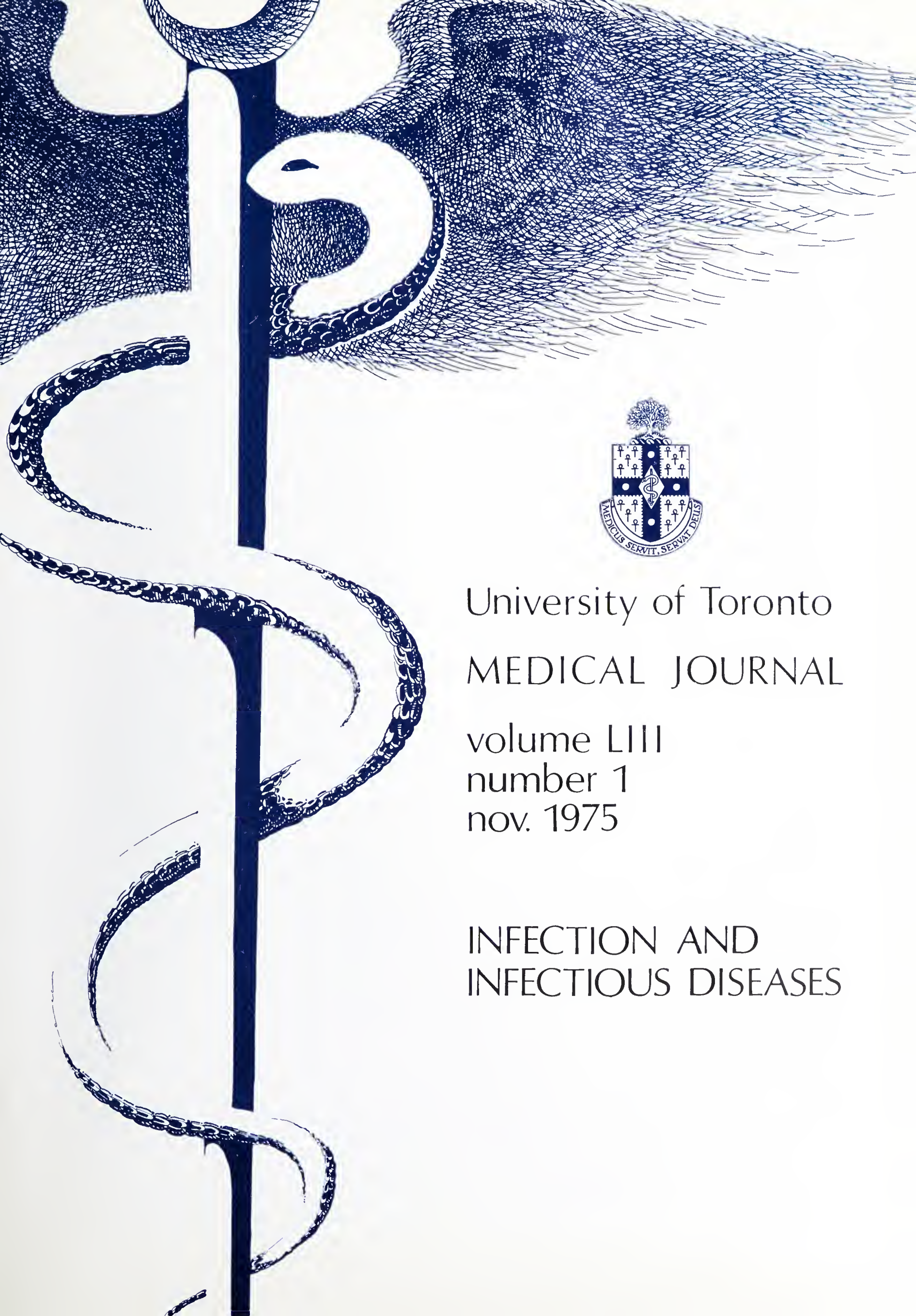

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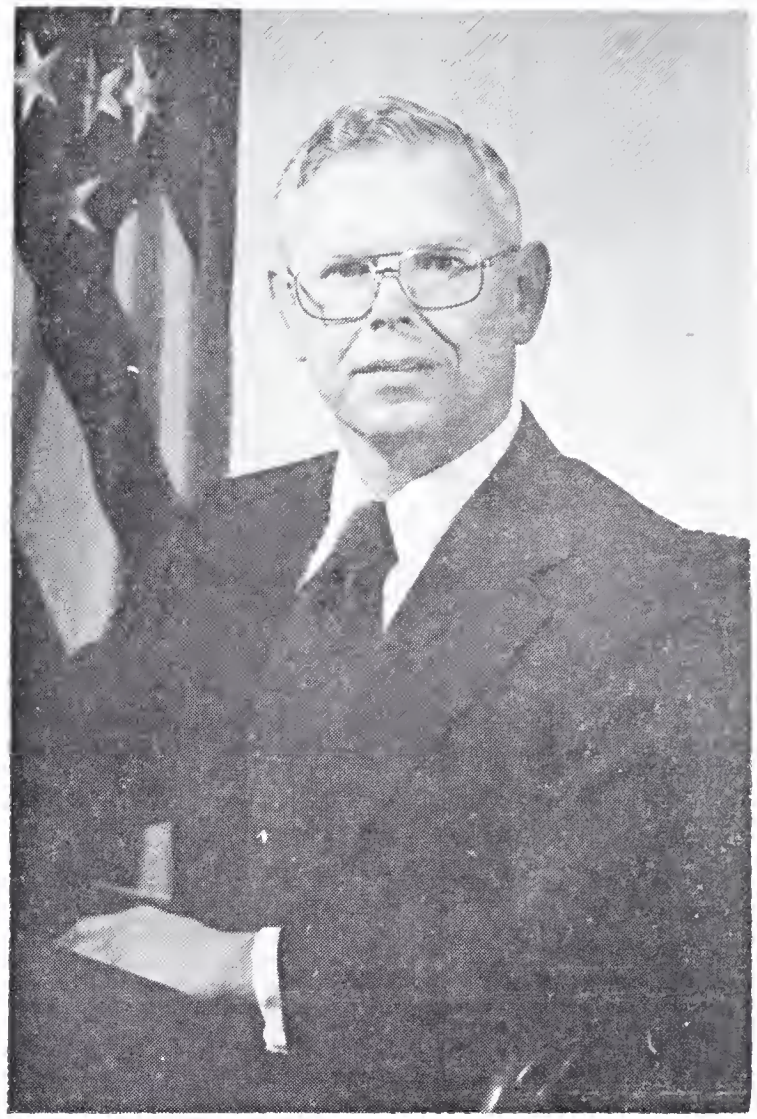

JAY SANFORD was born in Madison, Wisconsin May 27, 1928. He attended the University of Michigan graduating cum laude *in 1952. Dr. Sanford did post-graduate training in Medicine at the Peter Bent Brigham Hospital in Boston, Massachusetts and Duke University Hospital in Durham, North Carolina. He joined the staff of University of Texas, Southwestern Medical School in Dallas, Texas in 1957 as an Associate Professor of Internal Medicine and became Professor in 1965. Recently he has been named Professor of Internal Medicine and Dean, Uniformed Services, University of the Health Services School of Medicine in Bethesda, Maryland.*

Dr. Sanford has published over 150 articles and reviews and has been a contributor to several major text books, including Cecil-Loeb's Textbook of Medicine *and Harrison's* Principles of Internal Medicine. *He is an active member of an extensive number of committees and has been secretary of the Infectious Diseases Society of America since 1972. On November 13 Dr. Sanford was our guest lecturer at the Second Annual Medical Society Lecture.*

The Infectious Disease Specialist: Asset or Medical "Feather Bedding"?

At a time when medical practice is becoming fragmented into specialties, subspecialties and even sub-subspecialties, and when both the public and governments are questioning whether such specialization is justified in improving health care, or whether it is cost ineffective and essentially counter productive, the subspecialty of infectious diseases appeared. The specialty has flourished, as indicated by the growth of the Infectious Diseases Society of America from a nucleus of 50 persons in 1963 to approximately 1100 members today. Infectious diseases was recognized as a special area of certification by the American Board of Internal Medicine in 1972. Since then, 259 persons have been certified through two examinations. This growth has ensued at the very time when many consider the problems of infectious diseases to have been solved through the use of antimicrobial agents and vaccines.

We are faced, then, with two apparently disparate views, the one, that infectious disease problems are on the decline and medical subspecialization should be de-emphasized, and the other, reflected in the remarkable growth of the specialty. It is therefore appropriate to address ourselves to the following questions: (1) Have the problems of infectious diseases as causes of suffering, disability and death been controlled? (2) If not, what role should the specialist in infectious diseases assume, and what knowledge and skills should this individual have?

An answer to the first can be gained from a number of observations. An analysis of conditions for which patients seek the counsel of primary care physicians reveals that infections as a group represents a major cause. Studies on the epidemiology of urinary tract infections in South Wales have shown that each year one woman in five has symptoms of burning on urination and that one half of these individuals seek medical attention. Antimicrobial agents constitute the second most commonly prescribed group of drugs and, in hospitals, may consume as much as thirty to forty per cent of pharmacy budgets. Hospital associated infections occur in 3.5 to 5.0 per cent of all patients admitted to hospitals. In patients with haematologic neoplasms, or following organ transplantation, infection remains the major cause of death. While one could go on with examples, it should be clear to all familiar with medical practice – in either the ambulatory or hospital setting – that infections continue. However, since infectious diseases lacks an organ system on which to base the discipline, the majority consider these infections to fall within the realm of the generalist, the nephrologist, the chest physician, etc. This brings us to our second question. namely, What is the role of the infectious disease specialist and what special skill can he contribute to a medical problem? Training in infectious diseases requires the development of expertise in five major

areas: (1) clinical infectious diseases; (2) mechanisms of host-parasite interactions; (3) diagnostic microbiology including bacteriology, virology, parasitology and immunology; (4) epidemiology, control and prevention of infections; and (5) an understanding of the scientific method appropriate to the area.

Since infections involve all organs and body systems, the specialist in infectious diseases must be an accomplished generalist. This is perhaps one of the major attractions of the field. Infectious diseases is a non-specialized specialty. In many areas the infectious disease expert is utilized in a manner analogous to the consultant of the past, a physician's physician. The occurrence of an illness which may have been acquired in a remote area of the world is usually considered to fall into his realm.

The primary tool of infectious diseases is microbiology. The infectious disease specialist must be able to interpret the results of diagnostic tests in relation to clinical problems.

Knowledge of the methodology required for the surveillance and control of nosocomial infections should be a major area of expertise and commitment. Under this category will fall responsibility for Infection Control and Formulary (Pharmacy) Committees, established to assess patterns of drug use and misuse.

As Dr. Calvin Kunin has recently described, the role of the infectious disease physician is that of a hospital-based physician who is not only a consultant on difficult clinical problems, but is also responsible for assuring that the investigative efforts of the diagnostic microbiology laboratory are relevant to patient care, hospital infection control and antimicrobial usage. It is inappropriate to suggest that all infections should be treated by infectious disease specialists, but those of us in the rapidly emerging field, into which new antimicrobial agents are introduced almost daily, have the opportunity and the responsibility for improving the present prescribing practices.

It seems clear to me that there exists a need for the infectious disease specialist. While his role in an academic setting has been reasonably established, it has not been adequately defined for the community hospital, where the potential for significant contributions to medical care is perhaps the greatest. In Canada, where the types and numbers of specialists to be trained are regulated, it is important not to make the infectious disease specialist the fireman on a diesel engine, but rather to define how such specialists will be used, and from this to determine manpower needs. Those of us from south of the border will follow your efforts closely and with great concern, for we follow in your shadow.

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PUBLICATION GUIDELINES:

1) All articles intended for publication should be submitted to the editor before the deadline which is usually one month before the release of the Journal, i.e. for the November 15 issue, the deadline is October 15.
2) Submitted articles should be typed (or legibly written) double-spaced, single sided on paper 8½" x 11" (letter size) and they should not be more than 15 to 20 pages (3,000 to 4,000 words).
3) Illustrations, graphs, diagrams, figures and photographs should be put on a separate sheet and identified by Arabic numerals. A list of explanatory legends for all the figures and diagrams should be put on another sheet. Do not write on the back of photographs.
4) References should be checked with the original sources, designated by Arabic numerals in the text, numbered in order of appearance and put on a separate sheet. Each periodical reference should include in order: the author's name and initials, title of the article, journal name, volume number, page number and year (e.g. 1. ROSEN HJ: Lumbar intervertebral disc surgery: review of 300 cases. Can Med Assoc J. 101: 317, 1969)
5) The editor and associate editors reserve the right to make such editorial changes as are considered necessary to ensure correctness of grammar and spelling, clarification of statements and conformity to Journal style.
6. Acceptance for publication is subject to the understanding that such material is submitted solely to the Journal, and will not be reprinted without the consent of both the editor and author.

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Some Hazards in Antimicrobial Therapy

J. A. Smith, M.D., F.R.C.P.(C)*

There is probably no group of drugs with greater potential for both good and evil than the antimicrobials. Their life-saving benefits have been established beyond doubt; unfortunately, so has their ability to initiate or compound serious disease. Their use can adversely affect the microflora of the individual receiving them, but this is usually an acceptable price to pay if the antibiotic given is appropriate to the disease. It is also justifiable despite the change that large scale antimicrobial use can effect in the general hospital microbial environment. What is not acceptable is the use of antimicrobials which are not suitable for the disease being treated. The changes induced both in the recipient's microbial flora and in the total hospital ecology is too high a price to pay for ill-advised antibiotic usage.

It is against this background that the practitioner must determine when to use antibiotics and which agent(s) to use, as well as to have a clear understanding of the situations best managed by withholding antibiotics. Thus, it may be better not to prescribe antibiotics to a febrile patient who is not very ill when the cause of the fever has not been elucidated. On the other hand, a very sick febrile patient may best be treated by taking specimens for culture from a number of sites (blood, urine, throat, etc.) and then initiating empirical antibiotic therapy. This is a common approach to the management of a patient with a granulocytosis. Often, there is a choice of antibiotics available to us, and we may use combinations of drugs in some situations.

I have outlined some of the errors that may be encountered in the management of a few common infections seen in hospital. Some general rules apply to the use of antibiotics: (1) use only when necessary to reduce mortality or morbidity; (2) give in adequate dosages; (3) administer by the route appropriate for the disease; (4) give for the proper duration of time; and (5) do not use antibiotics to the exclusion of other measures such as drainage of pus, physiotherapy, etc.

Note that antibiotics do not directly lower temperatures; they kill or help to eliminate microorganisms which cause fever.

RESPIRATORY INFECTIONS

Lower respiratory tract infection is often either a reason for, or consequence of admission to hospital. The diagnosis of pneumonia is usually based on clinical, radiological and microbiological assessment. Unfortunately, some diseases other than pneumonia may present clinically as pneumonia, cause radiological abnormalities, and set in motion a series of investigations, including "sputum" culture, which may produce misleading information. If a patient is not producing sputum, it is possible that saliva may be sent to the laboratory. It is also possible that culture of such saliva might yield gram-negative organisms that had colonized the oropharynx. The assumption that these organisms were causing pneumonia might lead to the administration of drugs such as aminoglycosides to a patient with a neoplasm, atelectasis, pulmonary infarction or pulmonary oedema.

Clearly, a precise microbiological diagnosis can only be made if the appropriate specimen is sent to the laboratory. Thus, sputum, transtracheal aspirate or pulmonary tissue are acceptable; saliva is not.

Aspiration pneumonia is worthy of special mention as a pneumonia which is often misdiagnosed. Spillage of oropharyngeal secretions past the glottis into the terminal bronchioles and alveoli results in the establishment in lung tissue of many organisms including oral anaerobic bacteria, and possibly enterobacteriaceae such as klebsiella. This disease is seen not infrequently complicating anaesthesia while the stomach is full, with regurgitation and aspiration of stomach contents. This is most likely to occur before insertion or after removal of a cuffed endotracheal tube. Loss of consciousness because of alcoholic intoxication, hypnotics and narcotics may result in aspiration of oral secretions, as may neurological deficits such as stroke, loss of gag reflex or impairment of the swallowing mechanism.

Aspiration of oral secretions occurs most often when patients are lying supine. In this position, the most dependent segments of lung are the apical segment of the lower lobe and the lateral and posterior portions of the upper lobe.

When the pneumonia is well established, radiological examination may show a "mid-zone" infiltrate. At this time, sputum examination may reveal some pus cells and a mixture of oral bacteria on gram stain and culture. Enterobacteriaceae may be present. Should the pneumonic process progress to necrosis and abscess formation, the suspicion of aspiration may lead to a correct assessment of the pathogenesis of the pneumonia, and to the initiation of therapy which should include an antibiotic effective against most oral anaerobic bacteria; penicillin G is particularly appropriate. However, when the infection remains as an infiltrate without abscess, and if a preponderance of gram-negative rods are cultured, the clinical situation may be misinterpreted. It is common for such patients to receive aminoglycosides with or without cephalosporins, but poor response may raise the suspicion about inaccurate diagnosis. A complete re-evaluation of the history and clinical and laboratory data at this point may result in transtracheal aspiration of lower respiratory secretions in an attempt to define the illness with greater accuracy. The presence of squamous epithelial cells on gram stain will confirm aspiration pneumonia and should suggest the administration of penicillin G.

Aspiration pneumonia is a good example of a serious disease in which the use of certain wide-spectrum antibacterial agents is not an adequate substitute for precise diagnosis and specific antibiotics. In treating

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pneumonia, every effort should be made to tailor the antibiotic to the cause of infection.

It should also be recognized that physiotherapy to remove retained secretions is often a most desirable modality of therapy and may indeed be a good substitute for antibiotics.

URINARY TRACT INFECTION

Over-diagnosis of urinary infection may be based on several factors. Firstly, it is a common clinical experience to find febrile patients who complain of dysuria and do not have urinary tract infection. This may be seen particularly in female patients with pelvic sepsis. Accepting such clinical evidence at face value without bacteriological confirmation might result in the needless administration of an antibiotic. This is undesirable because the drugs are expensive, may cause side effects, and in this situation not only fail to benefit the patient but could confuse the clinical picture and deprive the patient of appropriate therapy. Another cause of over-diagnosis is the acceptance of laboratory culture results based on poorly performed laboratory work. Since many urine samples are initially contaminated, it is important that steps be taken to prevent these bacteria from multiplying in urine to the point where they might be thought to be significant (10^4 or more). This problem can be prevented by delivering the specimen to the laboratory quickly or by keeping it at 4°C until it can be delivered, or by adding a bacteriostatic agent such as sodium borate which can later be diluted on the culture medium, allowing the organisms to grow.

Most urinary infections are confined to the bladder so that one of the urinary antiseptics is sufficient therapy. Sulphonamides, nalidixic acid or nitrofurantoin are effective in most cases, and are preferred to expensive aminoglycosides or cephalosporins. On the other hand, evidence of pyelonephritis constitutes good grounds for administering antibiotics that penetrate well into renal parenchyma (e.g., ampicillin, cephalosporin, aminoglycosides, co-trimoxazole). Treatment should be for fourteen days and follow-up for about six months will detect any recurrence.

PELVIC INFECTIONS

Endometritis is an occasional complication of childbirth. It may be caused by any number of organisms which gain entry to the uterine cavity from the vagina. A potentially hazardous diagnostic manoeuvre in these patients is to send a vaginal swab to the bacteriology laboratory from which organisms other than those causing infection might be cultured. Clearly, this might result in the wrong antibiotic being administered with all the attendant unpleasant consequences. Every patient with endometritis should have a swab taken from the cervix or, ideally, from the fundus of the uterus. The passage of a speculum to obtain a good specimen under direct vision is amply justified by the enhanced accuracy of bacteriological diagnosis.

Anaerobic bacteria are common causes of endometritis. The presence on a gram-stained smear of gram-positive cocci or gram-negative rods resembling bacteroides species which fail to grow on aerobic media should be taken as presumptive evidence of anaerobic sepsis. Since these organisms may not grow in less than forty-eight hours, treatment should be initiated on the basis of the smear, pending culture results, but organisms on smear that resemble anaerobes should be considered significant whether or not they grow on anaerobic media.

Infection complicating childbirth or operative trauma to the genital tract may result in sepsis extending into the broad ligament and to the Fallopian tubes and ovaries; there may also be pelvic peritonitis. Sometimes an abscess may form and may require surgery. More commonly, however, an abscess does not form, and antibiotics, if properly selected, may be curative. Again, vaginal bacteriology may be misleading, and it may be necessary empirically to use antibiotics effective against all the common pathogens. The ideal method is to obtain clinical material directly from the infected area by needle aspiration or laparoscopy, but this practice is slow to gain acceptance.

Following vaginal hysterectomy, some oozing of blood is very common. Should this collect into a haematoma, the patient may develop a fever and antibiotics may be given unnecessarily. On course, if the haematoma becomes infected and initiates associated soft tissue infection, antibiotics may be required.

PERITONITIS

Faecal spillage either at surgery or as a complication of intestinal perforation results in infection by mixed bacteria including bacteroides species. The antibiotic treatment of this disease is often a cephalosporin and an aminoglycoside, but unfortunately these are not effective against *Bacteroides fragilis,* the predominant pathogen among the anaerobic gram-negative rods. Clindamycin or chloramphenicol or high-dose penicillin would be appropriate components of the antibiotic regimen together with a wide-spectrum aminoglycoside (e.g., gentamycin, tobramycin, amikacin).

Obviously, no antibiotic can substitute for surgical drainage of abscesses which often complicate peritonitis.

SEPTICAEMIA

This is a life-threatening condition which must be treated aggressively. It may occur as a complication of other disease such as pyelonephritis, abscess, cholangitis or other septic focus. Also, it may cause serious haemodynamic disturbances, of which renal failure is one of the most common and most serious sequelae. The aminoglycosides can be life-saving in the treatment of septicaemia. However, they are potent agents requiring cautious use in the presence of renal impairment. Unfortunately, all too often caution is carried to the point where therapy is inadequate. The answer is to monitor closely the antibiotic concentration in serum and adjust the dose accordingly. This will hopefully reduce the risk of toxicity while keeping the efficacy

of the antibiotic at a maximum. It is of interest that in the past the therapeutic error in the situation described was failure to modify the dose of aminoglycoside (especially kanamycin) in the presence of renal failure.

ENDOCARDITIS

Antibiotics play a key role in the management of endocarditis. It is generally recognized that antibiotics must be bactericidal and that a concentration gradient be established between serum and the centre of an infected vegetation to kill the infecting organism. It is not good enough to give a patient an antibiotic to which the organism is sensitive. It is desirable that the bactericidal titre of the patient's serum be at least one in ten, and that it be maintained for four to six weeks.

Some uncommon organisms may require extensive laboratory testing to find a suitable synergistic combination *in vitro* that can be administered with comparable synergy in the patient.

It is usual for laboratories frequently involved in the management of endocarditis to stock all bacterial isolates from blood cultures for weeks or months. This practice evolves from the desirability at times to test an organism causing endocarditis against many antibiotics, not only at the time of original diagnosis, but at any subsequent time when it may be necessary to alter the therapy.

There are a number of recognized indications for surgical therapy in endocarditis, and one of them is failure of medical therapy. It may prove disastrous to persist with antibiotic therapy alone when removal of the infected valve is indicated.

TABLE 1
ANTIBIOTIC SENSITIVITIES OF COMMON GRAM-NEGATIVE ISOLATES
MOUNT SINAI HOSPITAL, TORONTO
1974

	NUMBER OF ISOLATES	% SENSITIVE					
		AMPICILLIN	CEPHALOTHIN	GENTAMICIN	KANAMYCIN	TETRACYCLINE	CARBENICILLIN
ESCHERICHIA COLI	1140	74	84	99	94	52	/
KLEBSIELLA SPECIES	380	4	90	99	96	66	/
PROTEUS MIRABILIS	275	94	95	99	97	3	/
ENTEROBACTER SPECIES	136	20	18	100	96	61	/
PSEUDOMONAS AERUGINOSA	208	0	3	91	3	4	86

TABLE 2
ANTIBIOTIC SENSITIVITIES OF COMMON GRAM-POSITIVE ISOLATES
MOUNT SINAI HOSPITAL, TORONTO
1974

	NUMBER OF ISOLATES	% SENSITIVE						
		PENICILLIN	METHICILLIN	AMPICILLIN	CEPHALOTHIN	CLINDAMYCIN	ERYTHROMYCIN	TETRACYCLINE
STAPHYLOCOCCUS AUREUS	400	24	97	24	99	99	98	90
STAPHYLOCOCCUS EPIDERMIDIS	519	42	92	48	97	94	90	56
ENTEROCOCCUS	275	44	17	97	53	20	82	30
BETA-HEMOLYTIC NON-GROUP A STREPTOCOCCI	175	83	82	100	87	94	90	26

CONCLUSION

Antibiotics are most useful when the choice is based on an intelligent appraisal of the clinical setting and on the most precise bacteriological diagnosis possible. They are incapable of curing febrile illness not caused by an infectious process.

Patterns of infectious disease vary considerably from time to time, and there is a continuous process of change in the microflora causing disease. For this reason alone it is not advisable to equate antibiotic regimens with diagnoses, but rather, the choice of antibiotics should be based on a knowledge of infecting organisms.

Sensitivities of some organisms, such as group A *Streptococcus*, are fairly predictable. However, in the case of gram-negative rods this may not be the case (Table 1). It is, therefore, a good policy to obtain an idea of the sensitivity patterns of organisms in one's hospital (Tables 1 and 2). Then it will be possible to employ intelligent empirical treatment of patients in hospital, where gram-negative rods and *Staph. aureus* are frequent pathogens.

In keeping with what I have said earlier, it should be noted that the figures and percentages quoted in the table may be peculiar to that particular hospital, and that the epidemiology of infectious disease within this one institution is subject to continuing change. Therefore, we can expect that the distribution of sensitivity patterns may be quite different in five years' time.

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Clinicopathological Conference: Infective Endocarditis

T. D. Ruddy, 7T7
H. E. Schlosser, 7T7
E. Fisher, 7T7

PROGRESSIVE DROWSINESS AND HEMATURIA IN A 79 YEAR OLD MAN

PRESENTATION OF CASE

Mr. E. M. was a 79 year old male who had become progressively more drowsy and unresponsive in the few days prior to admission on April 19, 1973. He also developed hematuria during this time.

Past history revealed the presence of benign prostatic hypertrophy, treated with transurethral resection in 1967. In May of 1971 the patient suffered a cerebrovascular accident affecting the right side of the body. Since then he required an indwelling catheter and suffered recurrent urinary tract infections. After a three month stay in a rehabilitation unit he returned home with only slight improvement, and was cared for by his relatives. He was incontinent of faeces for some time prior to the last admission to hospital.

Physical examination revealed a white, bearded, elderly, dehydrated and emaciated man. Vital signs included a regular pulse of 80 per minute, blood pressure 130/68, respiratory rate 14 per minute, and temperature 38°C. He was edentulous with a dry coated tongue. There were bilateral arcus senilis. The right pupil was small, irregular and non-reactive. The left pupil reacted well to light. Fundi were not visualized. Examination of the chest revealed an increase in the anterior-posterior diameter with decreased air entry at both bases. No definite rales or rhonchi were heard. The heart was enlarged with an apical impulse in the sixth intercostal space at the anterior axillary line. There was a grade II/VI pansystolic murmer at the apex and lower left sternal border. There was also a prominent epigastric pulsation. No evidence of edema, heart failure or elevated jugular venous pressure was found. Abdominal examination was unremarkable and it was noted that he had an indwelling bladder catheter and had some hematuria. There was a bruise overlying his left deltoid with decreased motion in the left shoulder joint. (These findings might be explained by a fall two days prior to admission.) There was a scar on the right forearm and excoriation and erythema of the buttocks, with no evidence of bed sores. Neurological examination showed him to be awake but responsive only to pain. He cried out with pain when his arms were moved. There was ptosis of the right lid, and a right hemiplegia with clonus of the right ankle, and spasticity of the right arm and leg.

Investigations included a chest x-ray which demonstrated gross atherosclerotic changes in the great vessels and mild left ventricular hypertrophy. There was also a faint increase in density at the left lung base probably due to a small effusion. On admission his hemoglobin was 8 gms per cent with a hematocrit of 25, and a white cell count of 15,800 with 84 per cent neutrophils. The blood sugar was 152 mg per 100 ml. The BUN was 86 mg per 100 ml and electrolytes were normal. Serum creatinine was 2.8 mg per cent on April 21. Blood gases on April 22 showed a pH of 7.55, pO_2 126, pCO_2 16, and total CO_2 48.5.

On April 22 his temperature rose to 40°C and he appeared gravely ill. Clinically, it was thought that he might have aspirated into his lung with a subsequent pneumonia. He was initially treated with Ampicillin. Subsequent blood cultures revealed an *E. Coli* septicemia which was sensitive to Gentamycin, Colimycin, Kanamycin, and Chloramphenicol, but resistant to Tetracycline, Cephalosporin, and Ampicillin. He was placed on IV Gentamycin and also Keflin. He continued to run a high swinging fever and gradually lapsed into a state of unconsciousness. He died on the 6th of May at approximately 0830 hours.

CLINICAL DISCUSSION

In summary, this 79 year old gentleman presented with hematuria, progressive drowsiness, fever, a murmer, anemia, and a history of recurrent urinary tract infections associated with an indwelling urinary catheter. Fever and a murmer should suggest infectious endocarditis (IE). The presence of anemia, hematuria and a probable portal of entrance for organisms via the indwelling catheter make the diagnosis more likely. Blood cultures positive for *E. coli* established the diagnosis. Although the bacteremia was treated with an appropriate antibiotic, the condition of the patient deteriorated until death. Congestive heart failure due to valvular damage or myocardial destruction is the most probable cause of death.[1]

The development of all the clinical features of endocarditis can be related to four machanisms – *infection, hypersensitivity, embolization* and *metastatic infection*.[2]

Circulating antibody-agglutinins constitute an important element in initiating *valvular infection*. These agglutinins cause many bacteria to attach to a single platelet-fibrin thrombus forming a large inoculum capable of initiating infection. This is particularly important in the development of subacute infectious endocarditis (SIE) due to less virulent organisms.[2] The same concept also explains why only one to three per cent of cases of SIE are due to gram-negative organisms.[11] Although infections by such bacteria result in a high rate of bacteremia, *E. coli* has been shown to be a very poor stimulant of platelet agglutination *in vitro*, whereas *Staphylococcus* has been shown to be very potent.[4] Thus large aggregates of bacteria and platelets are readily formed with *Staphylococcus* and not with *E. coli*. These aggregates easily attach to damaged but sterile endothelium, often already a focus for platelet accumulation. An active infection, usually remote from the heart, such as that associated with an an indwelling catheter, results in an acute infectious endocarditis (AIE) because of the constant high degree of bacteremia with a resulting steady onslaught of "platelet-bacteria" inocula.

The anemia, weight loss, elevated temperature, malaise and rigours can be considered a constitutional reaction to the process infecting the valves. The more intense reaction of AIE correlates with the greater severity of valvular infection. Anemia is found in fifty

to eighty per cent of cases of IE and may progress quickly without apparent intravascular hemolysis. Fever is absent in five per cent of cases, particularly in those with azotemia.[11] Leukocytosis of ten to twenty thousand in common in AIE and less common in SIE, where it usually implies embolic phenomena or an infecting organism other than *Strep. viridans*.[2]

Acute infectious endocarditis results in much valvular destruction and thus murmers occur which may change rapidly and dramatically in intensity and quality. Early in the course of about one third of cases of SIE no murmer can be heard. Murmers may be absent in SIE although the disease process may have been in progress for weeks. The murmer may appear during or after treatment.[11]

Hematuria and uremia are common complications of infectious endocarditis. The uremia is often associated with little or no fever, negative blood cultures and the absence of hypertension.[11] Significant renal lesions occur in thirty-three to fifty per cent of cases of IE. These lesions include infarction secondary to embolization, focal glomerulonephritis and diffuse proliferative glomerulonephritis. Renal failure is usually due to diffuse glomerulonephritis. The development of both glomerulopathies is attributed to a host immune mechanism reacting to the valvular infection, and is not considered to be the result of direct embolization of infected material to the glomeruli.[5]

The persistent bacteremia of endocarditis gives it a singular place among bacterial infections. The continued presence of infecting organisms in the blood strongly stimulates antibody production and subsequently leads to the evolution of soluble circulating antigen-antibody complexes. These complexes would exist at least intermittently in the presence of antigen excess and may manifest as *hypersensitivity phenomena*.[6]

High serum levels of agglutinating antibodies, complement-fixing antibodies and opsonic antibodies have been found in patients with SIE.[7] Also, the titre of IgM anti-IgG antibody (rheumatoid factor) has been shown to be elevated during the course of SIE and lowered after successful treatment.[8] Immune deposits have been shown along the basement membrane of glomeruli of patients with SIE. Thus the immunologic mechanism of the glomerulopathies seems well substantiated.

The inflammatory response to deposits of immune complexes in the walls of small distal arterioles may explain the presence of Osler's nodes, Roth spots, clubbing and petechiae. Similarly, an inflammation of synovia may result in arthritis and arthralgia. Petechiae are most common, occuring in one study in twenty-six per cent of cases. In the same study Osler's nodes were found in twenty-three per cent of SIE and none in AIE. All of the hypersensitivity phenomena occur more in SIE than in AIE,[3] probably due to the longer course of the illness and the resultant greater production of immune complexes.

PATHOLOGICAL DISCUSSION

Vegetations of infective endocarditis were found at the base of the aortic valve cusp. E. coli was cultured from heart blood at autopsy.

Patients with IE have been divided into two distinct groups on the basis of age, infecting organism and clinical course. One group is typified by a thirty year old woman with rheumatic heart disease, her mitral valve infected by *Strep. viridans* and responding well to antimicrobial therapy. The other group is represented by a sixty year old man with degenerative heart disease, developing an infection of his aortic valve with an organism other than *Strep. viridans* which responds poorly to antimicrobial therapy and is fatal.[11] Our case is very much like the latter.

Predisposing factors associated with aging include atheromatous deposits on the aortic valve and calcification of the mitral annulus or the aortic valve.[11] *Lipid deposits and calcification were seen at the base of the posterior aortic cusp. The annulus of the mitral valve was normal.*

Mortality associated with IE is usually due to congestive heart failure, accounting for sixty per cent of of deaths from IE in this antibiotic era. Two thirds of these deaths are due to aortic cusp perforations and subsequent regurgitation.[11] The development of aortic cusp perforation during the course of IE should be recognized because of the poor prognosis associated with sudden onset of aortic insufficiency. The development of heart failure unresponsive to medical treatment is an indication for aortic valve suture or replacement.[10] In this case, *the posterior cusp was a little thickened and calcified but showed no other damage.*

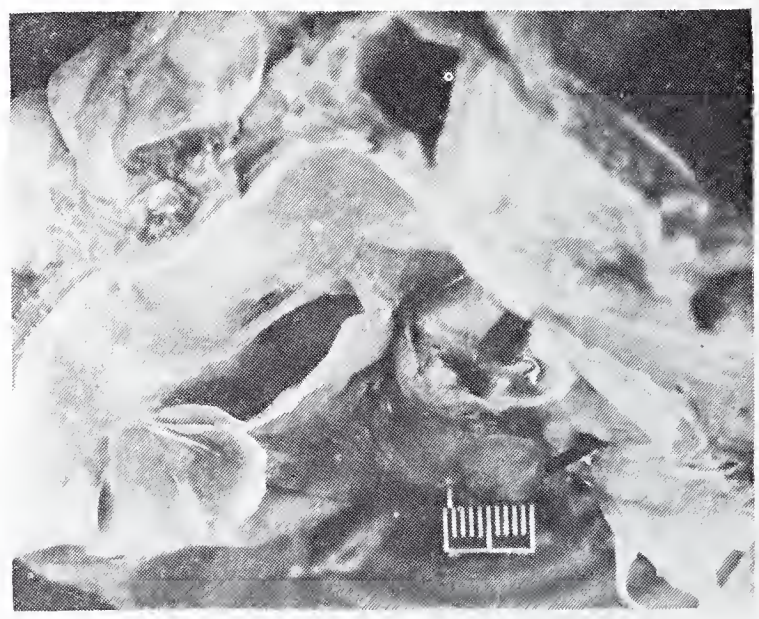

FIGURE 1
Aortic valve viewed from aortic aspect (left aspect to far left of photograph, right cusp in mid field and posterior cusp to right), showing infective endocarditis affecting posterior cusp. Infection spread from the vegetations of the cusp to cause 1) a ventricular septal defect (left black arrow); 2) a sinus of Valsalva abscess (middle arrow); and 3) infection on the ventricular aspect of the anterior mitral valve leaflet with perforation of that leaflet at its base (right arrow). (Scale indicates one centimeter.)

The infection extended downward through the base of the sinus of Valsalva to cause an abscess at the base of the cusp and involvement of the base of the intraventricular septum (Figure 1). Twenty per cent of patients with IE are found to have myocardial abscesses

at autopsy. They are not only the result of direct extension, but may also be due to dissemination of infected emboli from vegetations of the aortic valve along the coronary circulation, or focal lesions as a result of the bacteremia.[2, 11]

Myocardial abscesses usually involve the valve rings of the heart and surrounding tissue. This may be due to the relative avascularity of the valvular skeleton and consequent ease of growth for septic emboli, or merely the result of the frequency of direct extension of valvular lesions. Abscesses may develop in healthy or diseased hearts and are usually caused by *Staph. aureus* or gram-negative organisms of the urinary tract.[12]

Detection of such abscesses is difficult clinically. Suspicions should be aroused by persistent infection despite effective antimicrobial therapy, or by electrocardiographic evidence of conduction blocks or supraventricular tachycardia.

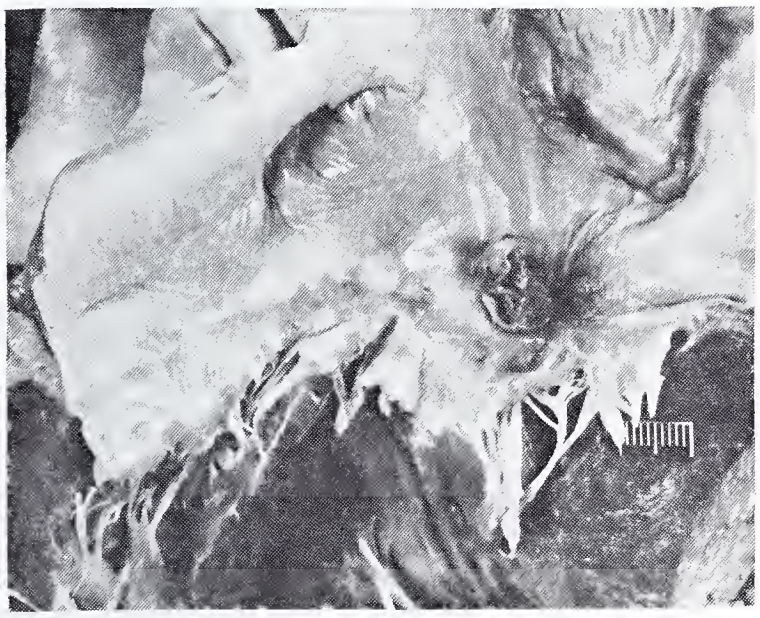

FIGURE 2
Perforation opening into left atrium and resulting from infection from the base of the posterior aortic cusp and base of anterior mitral valve leaflet. (Scale indicates one centimeter.)

In this case the infection spread to involve the base of the tricuspid valve and anterior leaflet of the mitral valve. Fistulae developed at the edge of the abscess passing from the outflow tract of the left ventricle into the right atrium, and through the anterior leaflet of the mitral valve into the left atrium (Figure 2). Such a development has a poor prognosis.[11] A clinical finding at this point would be the murmer of mitral insufficiency. A rare condition that would mimic such a finding would be a perforation from the left sinus of Valsalva into the left atrium, forming an aorto-left atrial fistula, leaving the mitral valve leaflets competent.[13]

Hyaline thromboemboli were swept into the coronary arteries causing a focal myocarditis and areas of myocardial necrosis. Myocarditis is considered to be one of the important complications of IE. Ischemic damage due to coronary occlusion by emboli may be complicated by the action of bacterial toxins, or inflammation due to the presence of immune complexes.[11]

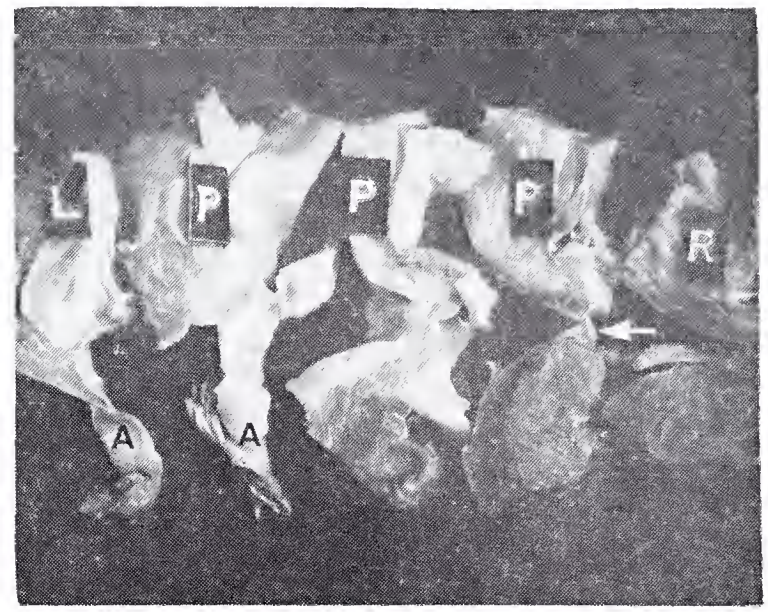

FIGURE 3
Proximal and distal relationships of the aortic annulus and sinus of Valsalva and structures likely to be involved if an infection (or aneurysm) spreads (L = left sinus, R = right sinus, and P = posterior sinus). Thus, distal spread may involve the aorta (rare), while proximal spread involves the left ventricular wall, the anterior mitral leaflet (A) or destroys the membranous interventricular septum (white arrow) and the adjacent conducting system.

There were very extensive areas of recent infarction involving both cerebral hemispheres. The majority of these lesions were aseptic, but some contained foci of cerebritis. Gram-negative rods were also identified in one of these lesions. This is not surprising since arterial emboli constitute the second most common complication of IE. The most frequent sites involved are the coronary vessels, spleen, kidneys and brain. Up to thirty per cent of patients with IE have maior cerebral emboli, usually in the distribution of the middle cerebral artery and its branches. Cerebritis or multiple microabscesses are the consequence of the inflammatory response to the deposition of infected emboli. The progressive drowsiness noted in the presentation of this case may have been caused by destruction of higher brain centres by repeated emboli, or the result of low perfusion due to the increased metabolic demands of the fever.

An old extensive deep-seated infarction of the left cerebral hemisphere correlated well with the stroke this patient had two years prior to his death. The urinary tract inflammation and chronic obstructive uropathy appeared relatively quiescent. The terminal bronchopneumonia was very minimal.

The presence of IE was an unexpected feature found at autopsy. Cause of death of this patient was cited as a pneumonia complicated by renal insufficiency. *The diagnosis of IE was not even suspected antemortem.* This is not an uncommon occurrence. In one series in 1940 it is reported that the correct clinical diagnosis of IE was made in all patients under forty years of age. However, in half of those over that age IE was discovered only at autopsy.[14] In a recent study at a large metropolitan Toronto "referral" hospital, it was shown that the clinical diagnosis was missed in 29.2 per cent of cases of IE arriving at autopsy.[15] Thus,

modern medical practice has made little apparent progress in diagnosing IE.

Many of the characteristic features may be less obvious or misinterpreted in the aged population. Fever may be absent or less conspicious. Coronary artery disease or arteriosclerosis of the valves or aorta is frequently considered to be responsible for all apical or basal systolic murmers. Heart failure is attributed to arteriosclerotic cardiac disease. Anemia is often ascribed to malnutrition. Neurological signs of sudden onset are usually regarded as the consequence of cerebral thrombosis and not of embolization. The elderly are more likely to get a valvular infection from an unusual drug-resistant organism. Two disease processes may and often do occur simultaneously.

The presence of a heart murmer and a fever of longer than one week's duration in a patient of any age is sufficient indication for investigation for infective endocarditis.[3] With early diagnosis, about eighty-five per cent of patients survive.[5] Without diagnosis, ninety-eight per cent of patients will die.[11]

ACKNOWLEDGEMENT

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Salmonella Gastroenteritis

*Hillar Vellend, M.D.**

Infections due to members of the genus salmonella, with exception of those due to *Salmonella typhi*, continue to occur at a rate which has not substantially changed in the last dozen years. It is estimated that approximately one-half to one percent of the population suffers from salmonella gastroenteritis annually. For these reasons, it is useful to review our current understanding of the epidemiology, pathogenesis and treatment of this disease.

MICROBIOLOGY

The genus salmonella belongs to the family enterobacteriaceae, which includes escherichia-shigella, klebsiella-enterobacter-serratia, proteus-providencia, arizona and erwinia. Salmonella are serotyped on the basis of their O- and H-antigens. There are over 1700 known *serotypes* and variants. These are grouped into *serogroups*. Ninety-five percent of human isolates fall into serogroups B, C and D; ten serotypes account for 70-80% of human disease. The commonest serotypes are shown in Table 1.

TABLE 1
MOST COMMONLY REPORTED SEROTYPES FROM HUMAN SOURCES – 1974

Serotype	*Serogroup*	*% of total*
typhimurium	B	31
newport	C	7
enteriditis	D	6
infantis	C	5
heidelberg	B	5
agona	B	4
typhi	D	2

EPIDEMIOLOGY

Although there is still intermittent controversy about the mode of transmission of salmonella, most sporadic cases and virtually all epidemics represent common-source outbreaks from contaminated food or water. However, person-to-person transmission can undoubtedly occur. Several factors contribute to difficulties in controlling dissemination of salmonella: (i) existence of a large animal reservoir, especially poultry (unlike *S. typhi);* (ii) mass production, rapid transportation and wide distribution of both animal feed and human food stuffs; (iii) unrecognized contamination of cooked food with raw food. Three recent epidemics serve to illustrate these points.

In early 1974, 80 isolates of *S. eastbourne,* a rare serotype, were reported from several constituencies in the United States and Canada. Most cases were in young children. The peak incidence occurred on December 29 and 30, 1973. Epidemiologic investigations implicated Christmas-wrapped chocolate balls. Almost all were manufactured by one company, a firm in Quebec which produced 14 million pounds of chocolate annually, with about a 50-50 distribution in Canada and the United States. Raw cocoa beans were found to be contaminated and when the manufacturing process was analyzed, the major fault was failure to separate storage areas for raw ingredients from finished product areas. Since most of the cases were widely separated, the outbreak probably would not have been detected if the serotype had been a more common one.

In the summer of 1975, six isolates of *S. florida,* a a very rare serotype, were reported from one community in Ontario. All had been patients of one physician – a gastroenterologist. All had undergone endoscopy and epidemiologic investigations implicated an inadequately sterilized fibre-optic gastroscope.

In the spring of 1972, 17 isolates of *S. agona* were reported from a small town, Paragould, Arkansas. All patronized a local drive-in restaurant. Cole slaw and raw onions were implicated, but the organism was also isolated from raw chicken. The cooler floor of the poultry distributor yielded *S. agona,* as did the environmental swabs at one of its major supplying slaughterhouses. The broiler flocks were fed with feed mill containing 8% Peruvian fish meal, which was contaminated with *S. agona.* Peru is a major exporter of fish meal, resulting in known outbreaks of *S. agona* in the United States, United Kingdom, Netherlands, Italy and Israel. This serotype, which was reported only twice in humans before 1970, is now the sixth commonest serotype isolated in the United States, with over 1000 isolates in 1974. Interestingly, the cole slaw implicated in the Paragould outbreak became contaminated because raw chicken was cut up on the same table that was used to chop ingredients for cole slaw.

PATHOGENESIS

It is useful to study the pathogenesis of an infectious disease in terms of organism virulence factors and host defense mechanisms against that pathogen.

NON-TYPHOIDAL SALMONELLA VIRULENCE FACTORS

These can be subdivided into (a) mucosal invasiveness, (b) extra-intestinal dissemination (lethal factors) and (c) fluid secretion (diarrhoea) mechanisms.

(a) *Mucosal Invasiveness of Ileum.* This property has been studied in several experimental models.

In preconditioned guinea pigs and germ-free mice, it has been observed that the epithelial cell microvilli degenerate when salmonellae come within 350 nm of its surface. This is followed by degeneration of the apical cytoplasm of the epithelial cell and eventually by ingestion of the organism through the brush border. The mediators of this ability to invade the mucosal cell are not known, but neither O-antigen, pili (as in gonorrhoea) nor flagellae are essential.

Mucosal invasion has also been studied in the isolated rabbit ileal loop. Virulent strains of *S. typhimurium* adhere to the brush border and invade the epithelial cells in 3-7 hours. Only about 5% of villi are invaded, mainly in the upper ⅓ of the villus and, in contradistinction to shigella, the organisms do not multiply within the epithelial cells and there is no epithelial cell destruction. Microabscesses, comprised of large num-

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bers of neutrophils, appear in the basilar portion of the villus, resulting in distortion of the overlying epithelium and depletion of goblet cell mucous. Clumps of bacteria appear in the lamina propria and some salmonella are found within neutrophils that infiltrate the lamina propria along with transformed lymphocytes. By 12 hours the bacterial infiltrate is widely scattered; by 18 hours, the villi are blunted and swollen, there is a massive infiltrate of neutrophils and the villous epithelium, although intact, becomes disordered into cuboidal and low columnar cells. Occasional organisms are seen within lacteals and within intraluminal neutrophils.

An *in vitro* Hela cell invasion model has been shown to correlate closely with this rabbit ileal loop model. The classical test for epithelial cell penetration utilizing the guinea pig cornea (Sereney test) is not positive, as it is for shigella.

The process of mucosal invasion has also been studied in the intact rhesus monkey, a natural host for this disease. The jejunum demonstrates minimal villous blunting but bacterial invasion is never observed. In the ileum, there is bacterial invasion of the mucosa, resulting in flattened villi, elongated crypts some of which contain microabscesses, edema and neutrophilic infiltrate in the lamina propria, and shortened, hyperchromatic epithelial cells. The colon demonstrates an acute colitis with multifocal microabscesses, mild epithelial cell disruption but only rare ulceration, and the lamina propria is edematous and massively infiltrated with neutrophils.

(b) *Extra-Intestinal Dissemination.* This is only observed with fully virulent strains. Most mice show dissemination to liver and spleen and 25% have positive cultures of heart blood. The O-antigen is important in salmonella virulence as measured by the LD_{50} after intraperitoneal injection in mice, but does not coorelate with mucosal invasiveness.

(c) *Fluid Secretion (Diarrhoea) Mechanisms.* Although fluid secretion has generally been attributed to mucosal invasion by salmonella, this is clearly an oversimplification.

When fluid secretion induced by various strains of *S. typhimurium* was studied in the rabbit ileal loop model, considerable strain to strain variation was observed. Fluid secretion started at 3 hours, before any morphological changes could be observed, and was maximal at 12 hours. However, they were unable to demonstrate any activity with cell-free extracts (i.e. a "classical" enterotoxin) in this model. Attempts to demonstrate fluid secreting activity by salmonella-stimulated neutrophils and sterile, aqueous extracts of infected mucosa failed. Thus they concluded that bacterial invasion was a necessary but not sufficient cause for fluid secretion.

The results obtained when net intestinal water, sodium and chloride transport was studied in the intact rhesus monkey are shown in figure 1. It was concluded that the process was not transudative since no net protein loss was demonstrated. The severity of the diarrhoea was related to the number of organisms in the lumen. The net fluid loss per unit length was greatest in the ileum. Jejunal fluid loss occurred without bacterial invasion. Although several mediators of fluid loss were postulated, including prostaglandins, none was demonstrated.

In 1975, a group of investigators published data in support of the thesis that strains of *S. enteriditis* and *S. typhimurium* produced an enteroxin. In order to understand this work, it is necessary to review briefly bacterial enterotoxins and experimental models used for their detection.

Toxigenic *E. coli* produce two distinct enterotoxins: a heat-labile (LT) toxin which is destroyed at 60°C and a heat-stabile (ST) toxin which is inactivated only after 30 minutes of boiling. LT resembles cholera toxin and is detected by its activity in the adult rabbit ileal loop and its ability to cause readily identifiable morphological changes in tissue culture systems such as mouse adrenal tumour cells. LT causes intestinal secretion by stimulating adenyl cyclase activity, resulting in elevated intracellular concentrations of cyclic AMP in the intestinal mucosal cells, resulting in increased secretion of sodium and chloride ions into the lumen. ST is detected by its ability to cause fluid accumulation in the small intestine of infant suckling mice.

Accordingly, supernatant fluid was harvested from exponential phase cultures of salmonella and tested in both the rabbit ileal loop model, which gave inconsistent results, and in the infant suckling mouse assay. The latter was quite sensitive provided certain methodologic precautions were observed: 3 to 5 day old mice were used; dilute preparations of the culture supernatant were employed, and the results were measured after an incubation period of 2.5 to 4 hours. The enterotoxin demonstrated appears to originate from the cell membrane (outer membrane fraction) and was closely associated with the lipopolysaccharide moiety. A comparison of the properties of the heat-labile (LT) and heat-stabile (ST) toxins of *E. coli* with the enterotoxin of *S. enteriditis* (SE) is shown in Table 2.

TABLE 2
PROPERTIES OF HEAT-LABILE (LT) AND HEAT-STABILE (LS) TOXINS OF E. COLI AND ENDOTOXIN OF S. ENTERIDITIS (SE)

	Assay Model		*Heat Resistance*		
Enterotoxin	Suckling Mouse	Adrenal Cell	60°C	80°C	100°C
LT	–	+	–	–	–
ST	+	–	+	+	+
SE	+	–	+	–	–

Host Defense Mechanisms Against Salmonella

These can be subdivided into (a) local gastrointestinal defense mechanisms and (b) systemic defense mechanisms. Either may be specific or non-specific.

(a) *Local Gastrointestinal Defense Mechanisms*

(i) Gastric Acidity. Bacteria instilled into the intact normal human stomach are promptly killed – in less than 15 minutes – at a pH less than 3, but remain viable in the achlorhydric stomach for at least an hour.

Gastric hypochlorhydria or achlorhydria (especially after gastric surgery) increases both susceptibility to and severity of gastroenteritis due to salmonella. In experimentally induced salmonella gastroenteritis in human volunteers, a smaller bacterial inoculum is required if antacids are taken simultaneously.

(ii) Normal Intestinal Motility. This appears to reduce the bacterial population of the jejunum and ileum and intestinal stasis promotes bacterial overgrowth. Use of agents that retard intestinal motility (opiates, Lomotil) can convert salmonella gastroenteritis to a bacteremic form of illness.

(iii) Normal Indigenous Intestinal Microflora. Shigella-indigenous flora interactions have been extensively studied and investigators have concluded that the low redox potential (Eh) and pH and the high short-chain volatile fatty acid concentration induced by the indigenous microflora significantly inhibit shigella multiplication. However, relatively little is known about the role of this interaction in the resistance of infection due to salmonella. Oral administration of streptomycin to mice has greatly increased their susceptibility to *S. enteriditis* infection and the ID_{50} of salmonella for germ-free animals is 10^1 compared to 10^6 in mice with normal flora. It was thought that the elimination of bacteroides from the intestinal tract correlated most directly with the enhanced susceptibility to salmonella infection. In human volunteers, pre-treatment with streptomycin can reduce by 1000-fold the bacterial inoculum required to produce disease.

(iv) Local Specific Immune Mechanisms (Gut-associated Immunity). These may act synergistically with ecologic control mechanisms. Intestinal IgA response to salmonella has not been studied but evidence from studies with *V. cholerae* and shigella suggests that IgA inhibits adherence of bacteria to mucosal surfaces. Interestingly, some bacterial species *(Strep. sanguis)* produce an IgA protease which cleaves intact IgA to yield Fabα and Faα fragments, possibly even further modifying secretory immune function.

(b) *Systemic Host Defense Mechanisms*

(i) Non-specific. General depression of resistance to infection as is seen with age, debility and diseases such malignancy (especially leukemia and lymphoma), alcoholic cirrhosis and collagen-vascular diseases, seem to predispose to salmonella infection, especially to bacteremia and focal extra-intestinal disease. Demonstration that corticosteroids and nitrogen mustard reduce the resistance of mice to *S. typhimurium* is probably relevant to humans. Systemic non-typhoidal salmonellosis appears curiously related to a number of seemingly unrelated diseases – namely viral hepatitis, louse-born relapsing fever, malaria, bartonellosis, sickle cell anemia and neoplasms that possess a hemolytic component. Weinberg has postulated that this predisposition is related to the hyperferremia associated with these conditions – either from liver cell or erythrocyte destruction. Experimental sideremia is known to cause a marked reduction in the inoculum size of pathogenic bacteria required to initiate a disease process.

(ii) Specific. IgG and IgM antibodies to somatic antigens of salmonella increase resistance to infection but their precise role is not known. Judging by the limited success achieved when these antigens are employed as immunizing agents, humoral immunity to at least these antigens is of limited importance. Cell-mediated acquired immunity is probably quite important but has not been studied in non-typhoidal salmonella infections.

TREATMENT

Except for serious bacteremic disease or focal extra-intestinal disease, there is no indication for specific antimicrobial therapy of salmonellosis. In fact, such therapy may have deleterious consequences by (a) promoting selection of multi-resistance in this germ, and (b) by prolonging the duration of intestinal carriage. These will be reviewed briefly.

(a) Resistance Transfer Factors in Salmonella. Resistance transfer factors (R-factors) are carried on extrachromosomal genetic elements called episomes (or plasmids). They confer upon the host organism the ability to transfer to susceptible organisms (by conjugation), genes which mediate antimicrobial resistance mechanisms. Isolation of increasing numbers of serotypes of salmonella resistant to multiple antibiotics (especially to such front-line agents as ampicillin and chloramphenicol) has led to studies examining the role of R-factors in this evolution.

Multiply-resistant strains of salmonella almost all have demonstrable R-factors and the overall prevalence of R-factors in the genus *Salmonella* is approximately 70%. The classical R-factor coding resistance to streptomycin, sulfonamides, tetracycline and chloramphenicol (Sm^R, Su^R, Te^R, Cm^R) is still relatively uncommon in non-typhi serotypes. Isolated reports of simultaneous resistance to both chloramphenicol and ampicillin (Am^R) is particularly disturbing. Although Am^R appears to be carried on a second plasmid, simultaneous carriage of both plasmids has been reported.

The selective pressures of exposure to antimicrobial agents in both man and animals seems to be the most important factor in this evolution. Acquisition of resistance may occur during antibiotic therapy of salmonella gastroenteritis.

(b) Intestinal Carriage of Non-typhoidal Salmonella. Untreated patients with symptomatic salmonella gastroenteritis gradually shed the organism, with an estimated "half-life" of approximately 2 weeks – i.e. 50% develop negative stool cultures by 2 weeks; 75% by 4 weeks; 87.5% by six weeks, etc. The rate of reduction of faecal carriage is reduced by treatment with ampicillin and increased in some patients by treatment with trimethoprim-sulfamethoxazole. It is estimated that 1 to 2% of patients become permanent carriers of non-typhoidal salmonella. Factors that may contribute to the chronic carrier state include advanced age and cholelithiasis.

These carriers should be managed by prohibiting them from occupations such as food handling and patient care nursing and by emphasizing personal

hygiene. Attempts to eradicate the carrier state have proven difficult. In those with gall bladder disease, cholecystectomy cures only 50 per cent. High dose ampicillin for several weeks may improve these results. There is some suggestion that treatment with trimethoprim-sulfamethoxazole may shorten the duration of faecal carriage, but this agent has not been systematically evaluated and its effectiveness is controversial.

In the rare instances where treatment is indicated, only three antimicrobial agents are consistently effective – ampicillin, chloramphenicol and trimethoprim-sulfamethoxazole – provided the organism demonstrates *in vitro* susceptibility. Despite *in vitro* susceptibility to such agents as cephalothin, tetracycline and gentamicin, they are not effective. The reason for this paradox may be related to the ability of effective drugs to penetrate macrophages.

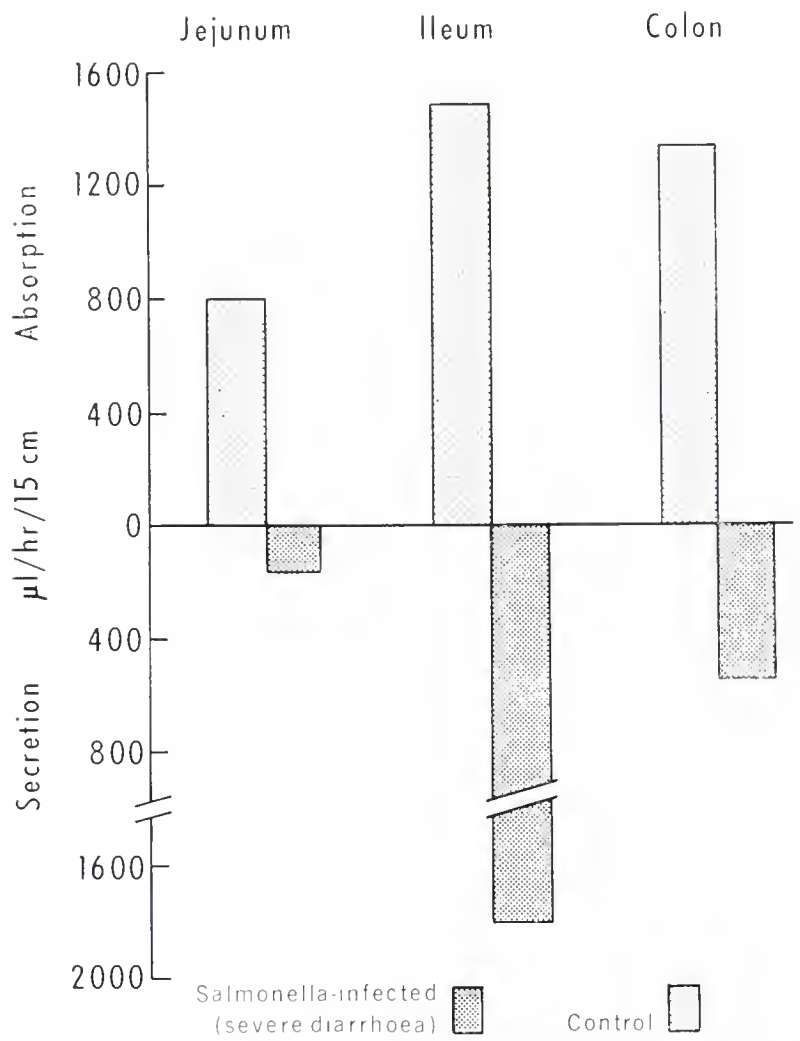

Figure 1: Net intestinal water transport in control and salmonella-infected rhesus monkeys. (Modified from Rout et al)

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Alkaline Phosphatase and Hypophosphatasia

Stephen P. Kraft, 7T8

INTRODUCTION

The subject of this paper represents a union of two areas of research that have been investigated with much interest in recent years. The precise roles that the enzyme alkaline phosphatase plays in various biochemical and physiological processes have yet to be elucidated, and hypophosphatasia represents a genetic disorder whose etiology still remains a mystery.

This article will review some of the background to each of these areas and will indicate how the enzyme may be involved in some of the clinical manifestations of the disease. Experiments done by the author during the course of a summer project in this subject will also be described and the results discussed.

ALKALINE PHOSPHATASE

Alkaline phosphatase (AP) is a general term for a heterogeneous group of enzymes that functions at alkaline pH to hydrolyze phosphate ester bonds. The pH optimal for activity ranges from 8.5 to 10.5 depending on which body tissue is being examined. For example, human renal alkaline phosphatase has its optimal pH at 9.4 with minimal activity at pH 6.0 and 11.0.[4]

Studies on chick embryos have shown that in early stages of development all cells contain AP. During intermediate stages, the activity of AP decreases in most tissues to undetectable levels. Later in development the enzyme action reappears in the organs and tissues which in the adult normally contain significant levels of AP.[2] These include the epithelium of the small intestine, the proximal tubule of the renal nephron, bone osteoblasts, granulocytes, hepatocytes, skin epithelium, active hair follicles, and even the placenta of pregnancy.[2] It is interesting to note that the alkaline phosphatases isolated from these various tissues show different electrophoretic mobilities.[11]

Histochemical studies have localized AP in cells to two areas. There is a diffuse cytoplasmic fraction which likely represents AP associated with microsomes. There are also deeply-staining granules which appear close to the cell membrane.[4]

Various established human cell lines show marked differences in AP activity, and variations of up to 500-fold among different tissues have been recorded. These differences are not related to cellular morphology nor to specific nutrient media used for culturing.[12]

AP is an *inducible* enzyme, and explants of intestinal tissue from birds, mammals, and amphibians can be stimulated to synthesize AP at various development stages earlier than the stages at which full activity usually becomes manifest.[2, 3] Such induction has been successfully demonstrated with both adrenal glucocorticoid analogues, such as prednisolone and dexamethasone, and with organic monophosphates, such as disodium phenylmonophosphate and beta-glycerophosphate.

As indicated earlier, bone osteoblasts show marked AP activity in both the adult chick and human. These are cells involved in the synthesis of the organic matrix of bone and they have high AP levels as evidenced by histochemical staining.[1] Various roles have been attributed to AP in the process of bone formation. Because this process is directly relevant to the understanding of various symptoms seen in hypophosphatasia, it is appropriate to briefly examine some of the theories that have been proposed about AP and bone formation:

1. AP may act on organic phosphate esters to hydrolyze the esters and liberate PO_4^{3-}. A high PO_4^{3-} concentration causes precipitation of $Ca_3(PO_4)_2$ which subsequently regroups into a complex bone salt known as hydroxyapatite.[1]

2. AP may be involved in the formation of the organic matrix of bone. Collagen fibrils are secreted by osteoblasts as PO_4 esters which are hydrolyzed off, possibly by AP, as the fibrils reach the cell surface. The fibrils recombine outside the cell to form fibres.[1]

3. Evidence exists that AP in bone is not truly a "hydrolase" but could be a "transphosphorylase". Studies have indicated that a type of phosphate ester associated with the matrix substance may act as a template to aid the crystallization of bone salt. AP may act to phosphorylate the required substrate to produce the ester that subsequently directs the deposition of calcium compounds to build up the characteristic crystals.

4. AP may function as a type of "housekeeping" enzyme. It may act to keep the bone substance free of certain phosphate compounds, such as organic pyrophosphates, that have been shown *in vitro* to be potent inhibitors of crystal growth in bone. AP thus hydrolyzes these potential inhibitors to allow growth of crystals.[1]

5. A new theory indicates that the osteoblasts themselves may govern the calcification process by synthesizing their own "calcification locus". This locus may be a cellularly-derived globule of a calcium phosphoprotein complex, and it may be put together in the Golgi apparatus of the osteoblast. AP would simply liberate PO_4^{3-} from extracellular sources once the complex is outside the cell, and these ions would combine with calcium ions on the complex to produce $Ca_3(PO_4)_2$ –like crystals which would subsequently rearrange into hydroxyapatite.[1]

HYPOPHOSPHATASIA

The incidence of this disorder is 1 per 10^5 live births, and the lesions to be described may develop in utero or at the time of birth.[6] The interest in this disease stems from the multitude of problems and curious symptoms that appear, and from the fact that the understanding of the etiology of some of these features may greatly advance our comprehension of various metabolic and physiological processes that are still unclear to this day.

1. Clinical Signs

The clinical signs of hypophosphatasia (HPH) are quite numerous. There are skeletal lesions in which the skull and long bones are affected, and one observes angulation of the long bones with marked kyphosis, muscle tenderness, and muscular hypotonia. There is defective calcification in preosseus cartilage and in recently deposited bone matrix. The zones of provisional calcification are reduced in size, and little mineralization is evident in the metaphyseal cartilage and in the secondary epiphyseal centres.[6]

Cranial abnormalities occur if the onset of the disease is in early infancy, and one sees a globular head, prominent eyes and a small head circumference, and the brain is enclosed in only a soft fibrous membrane leading to craniostenosis which causes severe CNS disturbances.[6]

Dental problems include premature loss of primary teeth due to inadequate alveolar bone growth and early resorption of teeth. Other clinical signs observed include anorexia, irritability, vomiting, anemia, dehydration, pyrexia, and failure of linear growth and weight gain.[6]

2. Biochemical Findings

Bone: The collagen structure of bone is disturbed and mineralization is abnormal. Marked changes occur at the growing regions of bone involving both membranous and cartilaginous growth.[6]

Kidney: Calcium salts become deposited in the tubular epithelium and in surrounding interstitial tissues. Renal function is impaired as evidenced by increased plasma urea levels, proteinuria, and decreased ability to concentrate urine.[6]

Calcium: Serum Ca^{++} levels are often elevated in severe cases but sometimes they can be normal. This may be due to increased absorption from the gut with little change in its rate of deposition in bone.[6]

Alkaline Phosphatase: The serum levels of AP are reduced to an average of 25% of normal and fluctuation around this value is quite marked. There is no relationship between the degree of reduction in AP activity and severity of the disease.[6]

Tissue levels of AP are also marked reduced. Activity is decreased at regions of bone growth and is low in the liver and kidney as well. It is interesting that the activity of AP from intestine is *not* decreased.[11]

This gross reduction in AP is a striking feature of HPH, and has been the chief focus of attention in the study of its etiology.

Phosphorylethanolamine (P.E.A.): This compound is involved in one of the metabolic pathways leading to synthesis of lecithin, and it is not detectable in the urine of randomly-selected normal adults. However, it is intriguing that there are detectable levels of P.E.A. in the urine of most HPH patients. This finding has also been observed in celiac disease, scurvy, and hypothyroidism.[6]

3. Genetics

There is no significant departure from a 1:1 ratio of female:male patients. Indications are that one quarter of the sibs of probands are affected with the disorder, and this fact, along with other findings, have led to a fairly convincing hypothesis that HPH is a monogenic disorder inherited as an autosomal recessive.[6]

4. Possible Etiology and Therapy

There have been a number of suggestions as to the nature of the disease:

a) Inhibition of osteoblast activity by some toxic factor

b) Hypersensitivity to Vitamin D

c) Disturbance of osteoblast activity during elaboration of the cellular matrix and/or at the level of the calcification of the bone matrix.[6]

Therapy with Vitamins D and C and with thyroid extracts has not proved useful. In one patient, oral cortisone showed promising results as it led to some mineralization where it was previously deficient, to higher AP levels in serum, and to lower calcium levels in serum.[6]

DISCUSSION OF SUMMER PROJECT

The discussion thus far on alkaline phosphatase, hypophosphatasia, and the features relating the actions of the enzyme to specify findings in the disease helps clarify the following portion. The work to be described took place over a six week period during July and August of 1975 and it was done by the author in the Medical Genetics Department of the Faculty of Medicine at the University of Toronto.

1. Objectives

The objectives of the experiments were as follows:

1. To compare the levels of AP activity in normal foetal and normal adult skin fibroblast lines.

2. To attempt to reproduce results described in earlier papers by observing if any changes occur in the AP activity of these skin cells under the influence of an adrenal glucocorticoid analogue.

3. To compare AP levels of adult HPH and foetal HPH fibroblast lines to the appropriate normal lines.

4. To observe whether a glucocorticoid analogue could significantly alter the AP activity of these HPH lines.

Unfortunately, the adult HPH line chosen did not grow well in culture and could not be used. Thus, the work was done with a normal adult line, a normal foetal line, and a foetal HPH line.

2. Methods

a) *Culturing Procedures* (See Figure 1)
Work done on each of the three lines covered a two-week period. For a given line, cells of generation G-03 (third generation) were placed

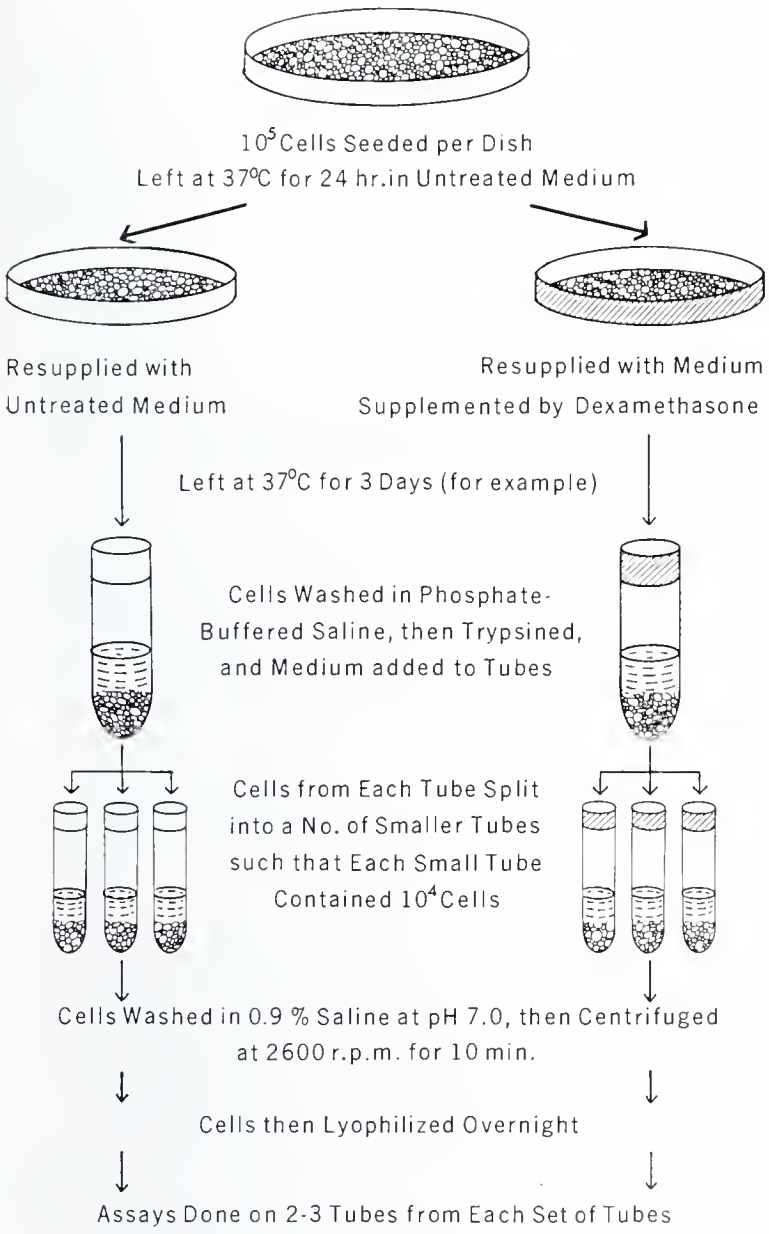

10⁵ Cells Seeded per Dish
Left at 37°C for 24 hr. in Untreated Medium
Resupplied with Untreated Medium
Resupplied with Medium Supplemented by Dexamethasone
Left at 37°C for 3 Days (for example)
Cells Washed in Phosphate-Buffered Saline, then Trypsined, and Medium added to Tubes
Cells from Each Tube Split into a No. of Smaller Tubes such that Each Small Tube Contained 10⁴ Cells
Cells Washed in 0.9 % Saline at pH 7.0, then Centrifuged at 2600 r.p.m. for 10 min.
Cells then Lyophilized Overnight
Assays Done on 2-3 Tubes from Each Set of Tubes

FIGURE 1

Procedures Used in Cell Culturing and Preparation of Cells for Assays.

The cells in each line used were incubated for 24 hours at 37°C before any cells received treatment with the drug. This allowed them to enter logarithmic growth phase. The steps shown were those followed for the dishes harvested on Day 3 of a typical week-long series of experiments: the same steps were followed for dishes harvested on any day during such a week.

into a number of culture dishes supplemented with minimal medium (consisting of 15% dialysed foetal calf serum and RNA, DNA, antibiotics and other nutrients) and at a concentration of 10^5 cells per dish. These were incubated for 24 hours at 37°C to allow the cells to enter logarithmic growth phase. On the following day, the medium was removed and the dishes split into two groups. One group was resupplied with fresh medium (the "controls") while the other group of dishes was supplied with medium supplemented with the glucocorticoid analogue dexamethasone at a concentration of 0.5 μg/ml. The two sets of dishes were reincubated at 37°C.

Analogues such as dexamethasone and prednisolone (see Figure 2) have been shown to cause maximal induction of AP by the fourth day after this seeding.[4] Thus, on various days for a week, cells were harvested from both the control and drug-treated sets of plates and prepared for subsequent assays (Figure 1 follows the procedure for the particular case of cells harvested at Day 3). From each dish harvested on a given day, a number of tubes with 10^4 cells per tube were prepared and lyophilized overnight to break open the cell walls.

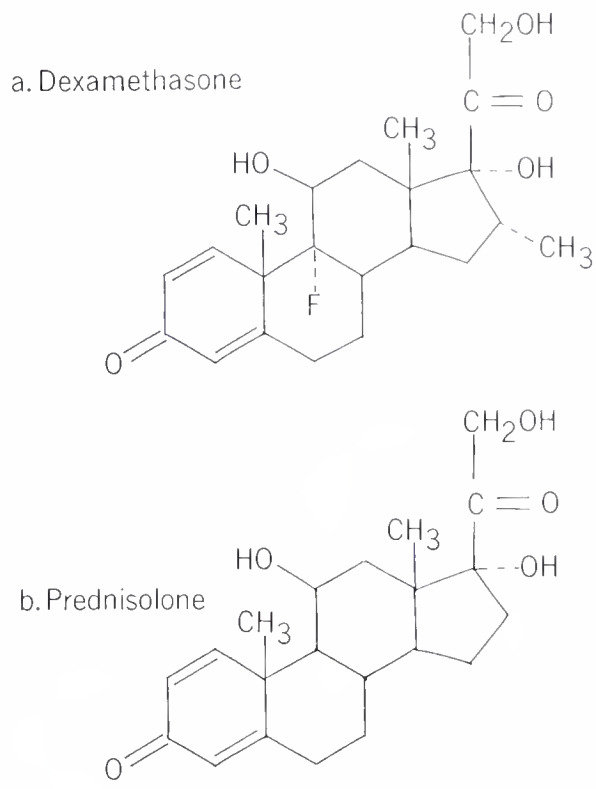


FIGURE 2

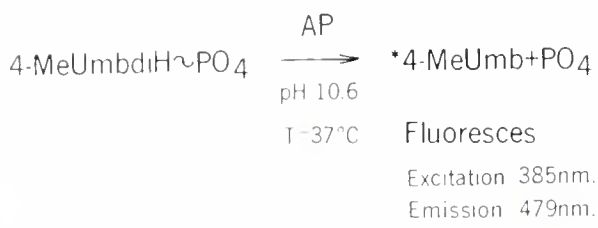
Structures of the Glucocorticoid Analogues Dexamethasone and Prednisolone.

The concentration of dexamethasone used in these experiments was 0.5 μg/ml.

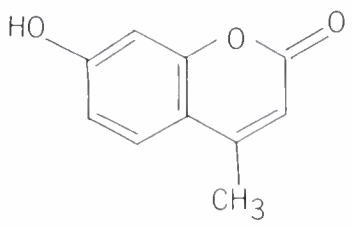
b) *Assays*

The following day, lyophilized tubes from each of the two sets of treatments were selected, and the material in them was resuspended in an amino-methyl-propanol buffer at pH 10.6. The assay tests were done at 37°C at this pH. Activity of AP was measured by fluorometry. The substrate used was a two-ringed compound related to coumarol (the synthetic anticoagulant) known as 4-methylumbelliferyl dihydrogen phosphate. AP acts to split off the PO_4^{3-} group and produce 4-methylumbelliferone. When this compound is excited, it fluoresces with an emission at a wavelength of 479 nm (see Figure 3).

The increasing fluorescence produced in a reaction was recorded in a fluorometer over a time interval sufficiently long to allow a steady linear activity to become obvious on a recorder chart. The fluorescent activity was calculated against a standard.



a. Alkaline Phosphatase Assay Reaction



b. *4-Methyl Umbelliferone
(7-Hydroxy-4-Methyl-Coumarin)

FIGURE 3

Structure of 4-Methyl Umbelliferone and Reaction Used to Assay for Alkaline Phosphatase.

The levels of product produced by the reaction were measured by fluorometric techniques.

The units of enzyme activity were calculated in terms of the number of nanomoles of product, namely 4-methylumbelliferone, produced per hour per 10^5 cells. Thus:

1 unit of activity is the amount of enzyme in 10^5 cells that can produce 1 nanomole of product from the substrate in 1 hour.

3. RESULTS

a) *Normal Foetal Line* (See Figure 4)

In Figure 4 the alkaline phosphatase activity is recorded (in enzyme units) over a six-day period for both the untreated cells and cells treated with dexamethasone. Also plotted is the cell number for dishes treated with and without the drug.

There seems to have been an obvious induction of AP activity after Day 3 by the drug, as the graph shows. Also interesting to note is the fact that the cells treated with the steroid did not divide as rapidly as the untreated ones, even though they were seeded at the same initial concentration.

b) *Normal Adult Line* (See Figure 5)

The same parameters are plotted in Figure 5 as were recorded in Figure 4, but it is obvious in this case that the drug caused *no* apparent induction over the six days. In fact, it seemed to have inhibited the AP activity to a slight degree.

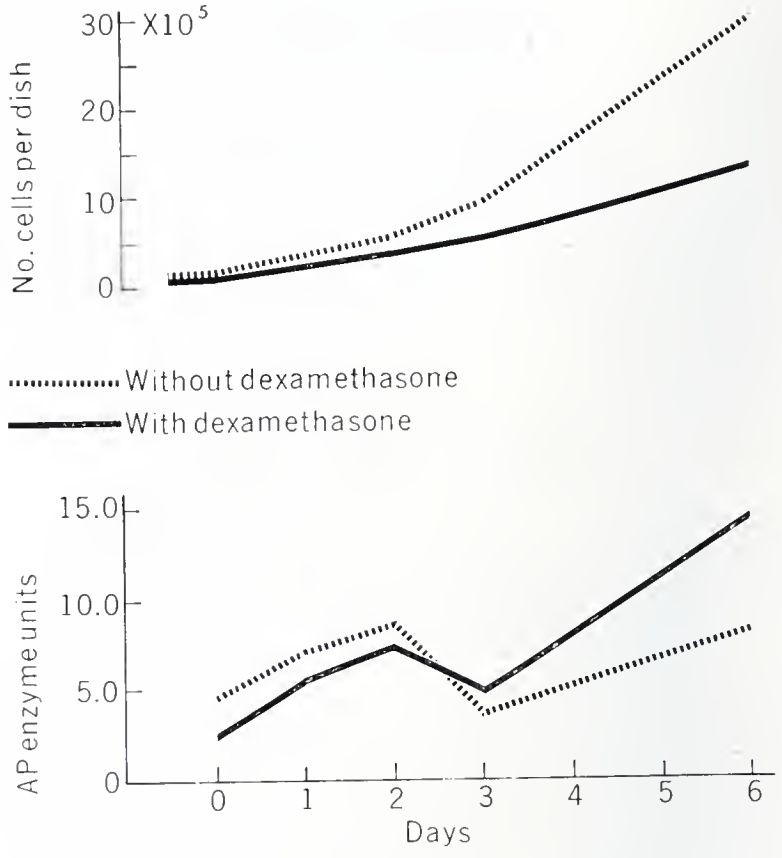


FIGURE 4

Cell Concentration and Alkaline Phosphatase Activity versus Number of Days of Incubation in Presence and Absence of Drug for *Normal Foetal Skin Fibroblast Line*.
Days are numbered from the time at which cells were first treated with the drug. Enzyme units are as defined in the text.

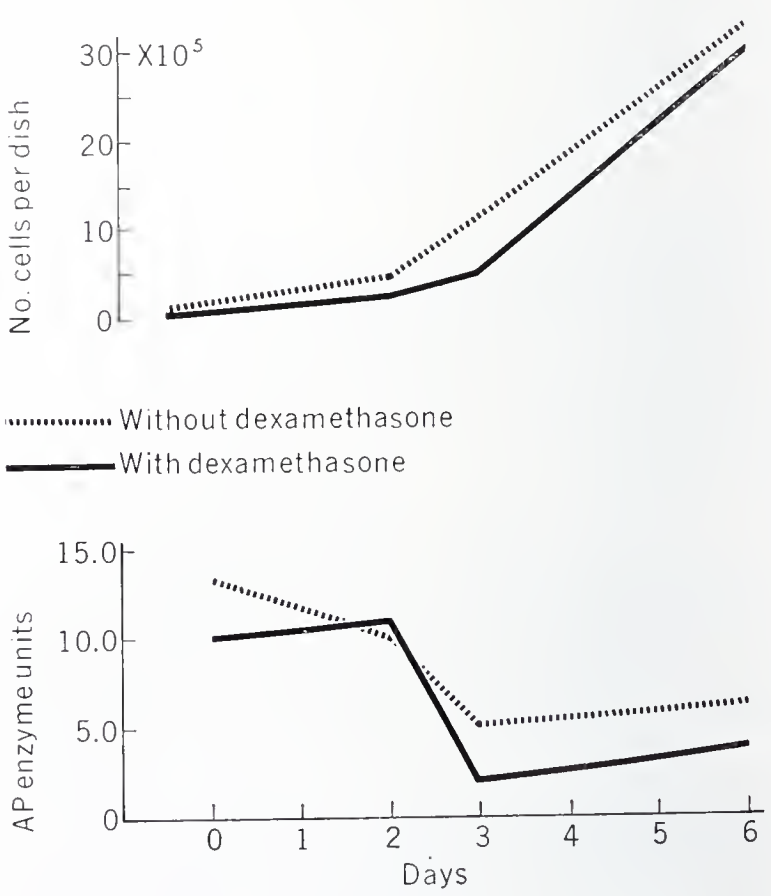


FIGURE 5

Cell Concentration and Alkaline Phosphatase Activity Versus Number of Days of Incubation in Presence and Absence of Drug for *Normal Adult Skin Fibroblast Line*.
Days are numbered from the time at which cells were first treated with the drug. Enzyme units are as defined in the text.

The cells without drug in their medium had a shorter lag phase than the drug-treated ones, but the rates of growth after this lag were similar and did not show the difference seen in the foetal line.

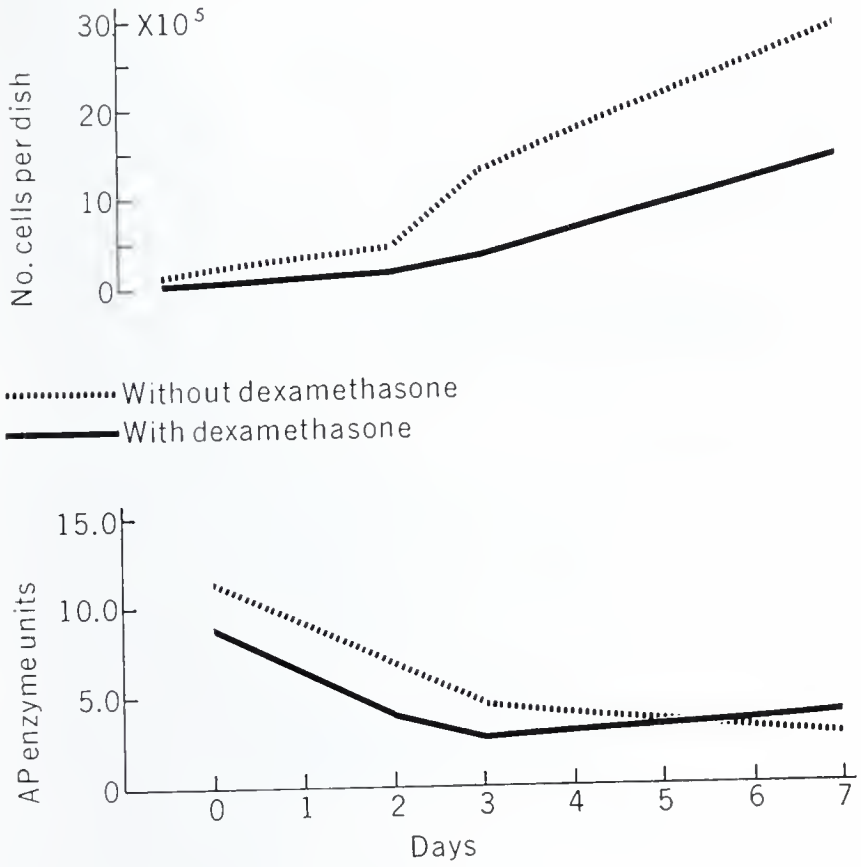


FIGURE 6
Cell Concentration and Alkaline Phosphatase Activity Versus Number of Days of Incubation in Presence and Absence of Drug for *Foetal Hypophosphatasia Skin Fibroblast Line*.
Days are numbered from the time at which cells were first treated with the drug. Enzyme units are as defined in the text.

c) *Foetal Hypophosphatasia Line* (See Figure 6)
The plot in Figure 6 indicates that there was considerable AP activity in this cell line at Day 0, but the activity fell with time in the control cells. The drug-treated cells, as in the normal adult line, did *not* show any evidence of induction by the dexamethasone. Again, the cells under the influence of the steroid did not divide as rapidly as the untreated ones.

4. DISCUSSION

The results with the normal foetal line brought to light two important facts. The glucocorticoid analogue can induce AP activity in certain cell lines, thus confirming a fact already reported by a number of investigators.[2, 3, 4] In addition these analogues cause a decrease in mitotic index, and along with this an increase in cell size and cytoplasmic volume.[3]

The adult line yielded some interesting information. Reports had indicated that *not* all cell lines can be induced by glucocorticoid analogues to increase AP activity and others may exhibit *very low* AP activity but can be induced by these compounds to synthesize much higher levels of AP.[4, 9] This adult line, not having been induced by the steroid, seems to be of the former type. Also, the comparison between the normal adult and foetal lines may simply reflect the fact that AP is present in all tissues in a foetus at high levels and can be readily induced by the analogues, whereas an adult has AP activity localized to specific tissues so that the fibroblasts of the skin, which have a low activity of the enzyme, may not be inducible by these compounds.

The foetal HPH results were extremely intriguing. These fibroblasts showed much AP activity, probably reflecting the sensitivity of the assay used, but, in contrast to the normal foetal line, showed *no* inducibility. This latter point is interesting since cortisone had been reported to reverse some of the phenotypic characteristics of HPH in one patient, and among these changes was the raising of serum AP levels.[6]

SUMMARY

It is obvious that much work remains to be done in the areas of both alkaline phosphatase and hypophosphatasia.

With regard to AP, it remains to be shown that a number of heterogeneous enzymes do in fact exist with possibly differing actions. This would seem reasonable since AP is localized in the adult to specific tissues encompassing a wide range of biological activities.

In particular, its precise role in bone mineralization, the deficiency which marks the most striking clinical feature of HPH, remains undetermined. HPH itself is still a genetic disorder with an etiology that has not been established conclusively as yet.

As a final point, the importance of the increased urinary excretion of P.E.A. in HPH and other diseases is unknown, and investigations into this problem may yield some interesting and significant information. It may lead to the development of suitable biochemical assays to detect heterozygotes or homozygotes for hypophosphatasia (for example, in utero) using P.E.A. levels as a supplement to tests for serum levels of alkaline phosphatase. Information from research into P.E.A. may not only lead to methods for detection and screening for hypophosphatasia, but could result in its use as a tool in the diagnosis of the other diseases in which urinary levels of P.E.A. are abnormally high.

ACKNOWLEDGEMENTS

This author wishes to thank Dr. David Hoar, in whose laboratory this project was done, for his many helpful suggestions, and the staff of the Medical Genetics Department at the University of Toronto for the use of their facilities and equipment.

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(continued on page 23)

Summer Scholarship Program

Sally McNamara, Awards Officer
Joseph Chu, 7T8

The aim of the Summer Scholarship Program is, broadly, to provide medical students with clinical and research experiences in environments which are conducive to learning. These experiences are to be complementary to the formal medical education and may serve either to satisfy the students' curiosity or to provide opportunities for possible career exploration. As the reports from both the supervisors and students who participated in the 1975 Program are assessed, it becomes evident that in these aims the Program has been quite successful. In addition, there have been other noteworthy results: in many instances the supervisors felt that the students were giving as much as they were getting either in terms of actively furthering the progress of an on-going research project, or exposing the supervisors to the trends of current medical education.

The Summer Scholarship Program has been maintained primarily through the generosity of such organizations as The Physicians' Services Incorporated (P.S.I.) Foundation, The Medical Alumni Association, The Medical Research Council and The Ontario Heart Foundation (see Fig. 3). The terms of reference of the various scholarships place certain restrictions on the selection of applicants and the type of work to be engaged in. For example, the P.S.I. Foundation, which funded 49 of the scholarships this year, stipulates that the scholarships be tenable in "clinical settings" in Canada, preferably in Ontario. The Medical Research Council requires that the students engage in research work at the University of Toronto and that they rank academically in the upper 20% of their class.

Last year 129 applications for Summer Scholarships were submitted for the consideration of the Awards Committee. Four members of the Committee (two students and two staff members) independently ranked the applications and then conferred with each other to produce a ranked list. The 91 scholarships were then recommended according to this list and with consideration to the stipulations of the awarding bodies.

SUMMER SCHOLARSHIPS – 1975
NO. OF APPLICATIONS

1st Year	Clinical or F.P.	19	58	129
	Research	39		
2nd Year	Clinical or F.P.	54	71	
	Research	17		

FIGURE 1

SUMMER SCHOLARSHIPS RECOMMENDED
1975

1st Year	Clinical or F.P.	17	42	91
	Research	25		
2nd Year	Clinical or F.P.	40	49	
	Research	9		

FIGURE 2

As shown in Figures 1 and 2, a few more applications were received from second year than from first year students. It is interesting to note that, no doubt as a result of their clinical exposure in Period IIA, about 70% of the second year students applied for family practice or clinical positions whereas the first year applicants, without this exposure, tended to show more interest in the research positions. The awards consequently bore out this application distribution and a greater proportion of second year students received the P.S.I. Scholarships, while the majority of the research positions went to students in the first year. Of the 57 clinical or family practice positions available, 40 went to second year and 17 to first year students; of the 34 research positions, 25 went to first year and 9 to second year students.

In evaluating the application the Committee members attempted to assess the educational potential of the proposed project. Consequently, a position emphasizing such routine duties as cleaning test tubes or office filing was given low priority. Applications for positions abroad were also given a low priority largely as a result of the restriction placed by the P.S.I. Foundation on their 49 scholarships. Except where academic excellence was a condition of the award (i.e., The Medical Research Council Scholarships) the relative academic standings of the students were not used in assessing the applications unless a student was required to do remedial work over the summer. In this event it was deemed inappropriate to award a Summer Scholarship since the work involved on the project would probably interfere with the remedial studying and vice versa. Since the emphasis of the scholarships is on educational value, previous experience in the proposed area of work was not regarded as necessary, although an application involving continuation of work begun previously was looked upon favourably.

DISTRIBUTION OF SUMMER SCHOLARSHIPS
1975

P.S.I. Foundation – Clinical Settings in Canada	($1,500)	49
Medical Alumni Assn. – Research	($1,200)*	9
Clinical or F. P.	($1,200)*	8
Medical Research Council – Research at U. of T.	($1,200)*	15
Ontario Heart Foundation – Research – Cardiovascular	($1,500)	3
Bradshaw-Errington	($1,200)*	4
James H. Cummings	($1,200)*	2
Ciba-Geigy – Research in Canada	($1,200)	1
TOTAL		91

* If possible these amounts will be increased to $1,500 in 1976 although this may decrease the number of scholarships available.

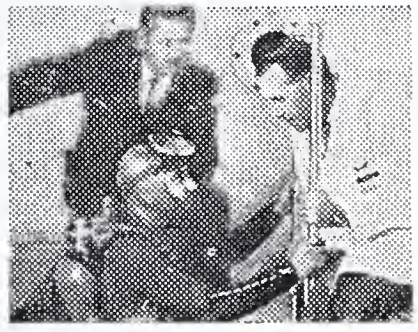
FIGURE 3

APPLICATION PROCEDURES

A list of potential supervisors will be compiled by the Awards Officer and made available to students just prior to the Christmas break. It should be emphasized that students are by no means restricted to the suggestions in this booklet and are encouraged to seek out their own supervisors and projects. Application forms will also be available in early December. These are to

(continued on page 23)

Your years at Medical School are the hardest. So how about some help along the way?



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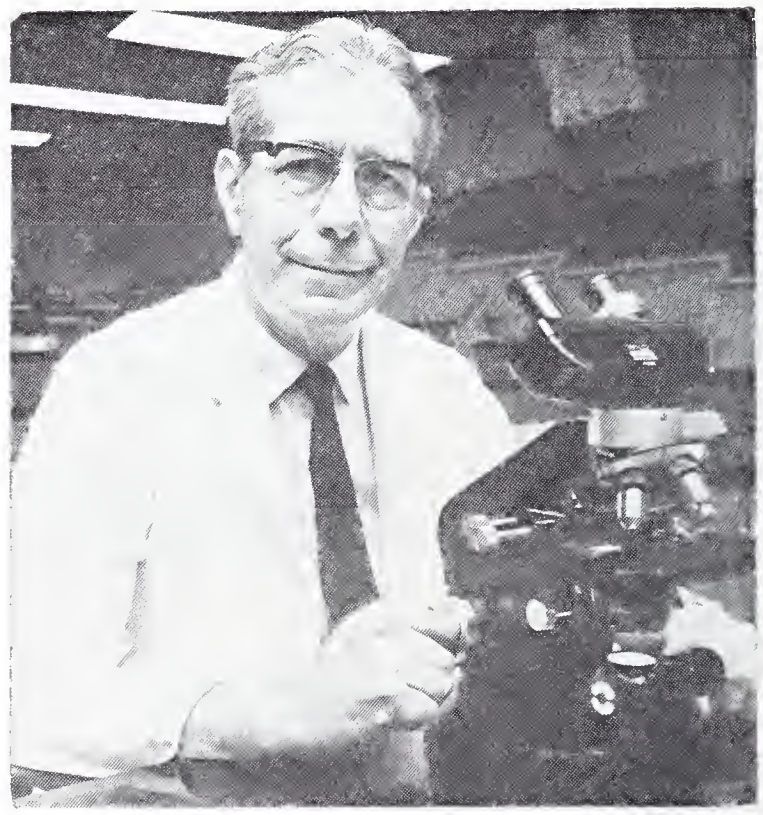
THE CANADIAN ARMED FORCES

DRS-70-21

Dennis Forrester, 7T8
Ken Roberts, 7T8

An Interview with Harry Whittaker



During the past summer, Harry Whittaker, Lecturer in Histology, received a 1975 Teaching Award from the Ontario Confederation of University Faculty Associations (OCUFA). The OCUFA Teaching Award Programme provides up to 20 annual awards in recognition of "established excellence in teaching" as an incentive towards "improving the general quality of university teaching". This award, therefore, pays tribute to the 45 years Mr. Whittaker has devoted to teaching and research in the Department of Histology. During this time he worked with many different faculty members, including Dr. J. C. B. Grant (author of *Grant's Atlas of Anatomy*), and Dr. A. W. Ham (author of *Ham's Histology* text). He is the mainstay of the Histology laboratories for the first year medical students, in which he is the senior demonstrator. He looks after the microscopes and projectors, and has himself prepared most of the slides that the students use. He is, as well, a specialist in the preparation of bone specimens for microscopic examination.

Harry's devotion to teaching can be seen in his unique style of presentation, which is simple, clear and highly instructive. His patient and easy-going manner has undoubtedly calmed the minds of thousands of first year medical students. One sign of his concern for students is his remarkable memory of the events over the years in which he has been teaching in the faculty. In talking with him, one gets the impression that he could name most of the members of the last 40 classes, and, in many instances, he can actually trace their careers, which he has followed "through the grapevine". His popularity with students has always been high. One example of this was his election in 1956 to an honorary membership in the Medical Society, a distinction which has been accorded to only a few selected individuals. Student initiative was a major factor leading to his nomination for the OCUFA award, which was endorsed by more than 600 students, and by both teaching colleagues and administrators.

D.F.: Do you enjoy teaching medical students?

HARRY: There is nothing else I'd rather do. I enjoy it very much. It's very stimulating to be around young people. It keeps you young in mind and spirit.

D.F.: Do you teach any students other than medical students?

HARRY: Yes. I also teach Arts and Science students and Pharmacy students.

D.F.: Have you noticed any difference in teaching these students compared with medical students?

HARRY: Well, I think the medical students are probably more aware of the significance of what they learn in Histology. The medical students accept the fact that it is part of their training even though they are not always aware of the full relevance. The other students tend to regard it more like another credit.

D.F.: What changes have you noticed in the medical school or in the students during your years on the teaching staff?

HARRY: I feel that the students coming in are more knowledgeable now and have a great deal more information. Otherwise, I haven't noticed any great change from one year to another – it's the same usual cross-section of people.

D.F.: What was the Histology course like back in the "old days"?

HARRY: The course was spread over the whole year, so it was less strenuous, but the total number of hours was about the same as it is now. There was also more emphasis on microscopic work then. Of course, there has been so much learned through research that the factual book-knowledge has become much more important today than recognition of structures under the microscope. In other words the swing has been from microscopic anatomy to Histophysiology. I also think that the Electron Microscope has changed the whole picture of things. We use to spend hours doing special stains and looking at little dots under the light microscope, which we thought were mitochondria, but didn't know what they looked like. It was really very frustrating at times.

D.F.: How well do you think students retain what they learn in Histology?

HARRY: The students retain more than they realize. I think that later on they'll probably apply it subconsciously. But there must be something there that we can build on all the time. The background information is required to help us understand more complex things as we go along. I remember talking to a student who

graduated a few years ago. I asked him what he had learned in Histology over the years in Medical School. He said that all he had learned was that there were two kinds of tubes in the body – epithelially lined and endothelially lined. I said, "Well, at least you have something there you can build on".

D.F.: Have you ever considered studying medicine?

HARRY: Not really. I don't think that I have the temperament suitable for the practice of medicine.

D.F.: Do you have any hobbies outside of school?

HARRY: I am interested in photography. I also have interest in Model Railroads in which I do a lot of reading. I really enjoy studying the history behind various railroad projects.

D.F.: You usually address the medical students as "Dr.". Would you like to comment on this?

HARRY: You are going to be a doctor unless something drastic happens – so why not? It gives you a little boost at this point.

D.F.: How do you feel about the Award which you recently received?

HARRY: It was one of the great thrills of my life, and I think it was very significant to me because it was initiated by the students themselves.

D.F.: What are some of your most treasured memories?

HARRY: Well, the fact that I met a lot of very nice people who have remained friends for many years. I enjoy seeing the students I have known become very important people in their fields. So I guess you could say my most treasured memory is seeing embryonic doctors develop into very capable and important men in their fields.

D.F.: Harry, you are quite famous among the medical students for your little gems, like "You don't have to be a mechanic to drive a Volkswagen".

Would you like to leave a final work of advice with the medical students?

HARRY: Yes. Remember, ladies and gentlemen – keep it simple. I think that's the key – simplicity.

(continued from page 19)

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(continued from page 20)

be completed both by the student and the supervisor and returned to the Student Affairs Officer by February 1st, 1976. Applications submitted after this deadline may not be considered.

The supervisor may use any method he/she chooses to select a student or students from applications, but is asked that he/she be restricted to the nomination of only that number of students for which facilities are available. Each student may submit only one application.

Editor's Note: If space permits, future issues of the *Medical Journal* will publish some of the students' reports/papers from the Summer Scholarship Program 1975.

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Book Review

Charles Krieger, 7T8

BRAINS AND REASONING
Gerhard Wassermann
165 pages. Cost: $21.50
MacMillan Co. of Canada Ltd.

"Science can contribute to an understanding of its own foundation by elucidating the brain processes which man uses in acquiring scientific knowledge."
– G. WASSERMANN

"A hypothesis is a little dog to sniff out truth with."
– MAXIM GORKI

Gerhard Wassermann, in his book *Brains and Reasoning*, proposes as his central thesis that "a neuropsychology-philosophy feedback cycle can be established which, by successive approximations, may provide gradually improving simultaneous answers to major problems of neuropsychology and many branches of philosophy, including the philosophy of science." Rather than regarding the philosophy of science and in particular the nature of scientific theorizing as something static and immalleable, it has become increasingly evident that scientific hypotheses are inherently limited, and to a certain extent stereotyped, by the source of their creation – a human mind.

As an example of this, approximation procedures (such as quantum mechanics and electromagnetic theory) as opposed to deductive theorizing (where rigid sequential logical operations from initial hypotheses to empirical generalizations must occur) frequently necessitate the use of decision or evaluation to determine the extent of the approximation required to 'fit' initial hypotheses to empirical generalizations. Thus in approximation procedures one is faced with the utilization of non-logical brain functions, e.g., evaluation, in the determination of scientific theory. This leads to concern for the particular mental or neural mechanisms that underlie this aspect of theorizing. Thus an understanding of the way scientific theorizing is accomplished alters the conception of scientific theorizing by the method of successive approximation.

"In zero order approximation we may consider man as a 'black box' who, by neuroscientifically unspecified neuropsychological processes, can generate hypotheses, invent deductive calculi and approximation procedures for these calculi, and who can evaluate results obtained by these and other methods. This zero order approximation cannot attempt to justify anything, but merely serves to pinpoint the problems and to characterize common aspects of scientific theorizing. It classifies those practices of scientific research which are widely shared by many sciences.

In the first order approximation we can no longer regard man as a black box. Instead, we must propose theories which suggest how brains could execute and physically represent all kinds of cognitive processes, since these are utilized in scientific theorizing. These theories, for instance, will have to explain how brains can represent concrete 'images' of visual configurations (and 'translate' these, via motor neurons and muscles, into diagrams)."

Dr. Wassermann claims that even "formal logical arguments as well as empirically-bound thinking all depend on brain-representations which have empirical antecedents." The book describes the inutility of a zero-order approximation to an understanding of decision procedures, the undecidability of 'reality problems', and the limitation of scientific theories, as well as languages and language usage. As an example, one of his conclusions is that "problems whose answers cannot be decided by a feedback between appropriate scientific theories and philosophy are purely metaphysical: they can be discussed only on the basis of arbitrary personal opinions. In contrast to this, presumptive decidable philosophical problems can, in my opinion, be approached only by the feedback cycle linking philosophy with neuropsychology and other sciences." Therefore, successive approximations on the neuropsychology-philosophy feedback cycle proceed asymptotically to an understanding of objective reality.

In chapters that follow, a thorough attempt is made to illustrate the necessity for the Wassermann method of successive approximation. A chapter concerning empirical statements and generalizations explains the relationship between public observation and the development of empirical generalizations, where empirical generalizations consist of hypotheses constructed on the basis of empirical observation. The remaining two chapters of the book discuss in detail the development and structure of theories from initial hypotheses through empirical generalizations and theoretical approaches to the life sciences.

The book (especially the middle chapters) is difficult to read in the sense that some conclusions are almost self-evident, and regardless of the great number of examples used from virtually every field of science, it tends to be verbose. Frequently, unnecessary complications arise by the use of detailed mathematical terminology. Although a mathematician by profession, Dr. Wassermann could still have attempted more readily understood verbal explanations.

There exist, however, many interesting observations. For example, concerning psychoanalytic theory: although it "has claimed to be 'mechanistic', it fails to specify any underlying processes explicitly. Those who criticize the theory are said to be victims of some 'complex' which forces the criticism upon them as a defence against 'id' forces." Or, concerning qualitative decisions: " . . . more generally, brain-models could only explain the range of cognitive and behavioural performances which nervous systems can control, and how this control could be effected. However, brain-models could never justify that use which brains make of their inbuilt mechanisms." Or, concerning consciousness: "It is also possible that the 'true carriers' of conscious experiences are not the neurons per se but particular elementary particles which are associated with physical fields which only become bound to particular proteins of specific neurons. If this purely speculative conjecture were correct, then these field-associated particles might become selectively activated through prior activation of the specific proteins to which they are bound, and the ensuing conscious state could be an epiphenomenon of the field activation."

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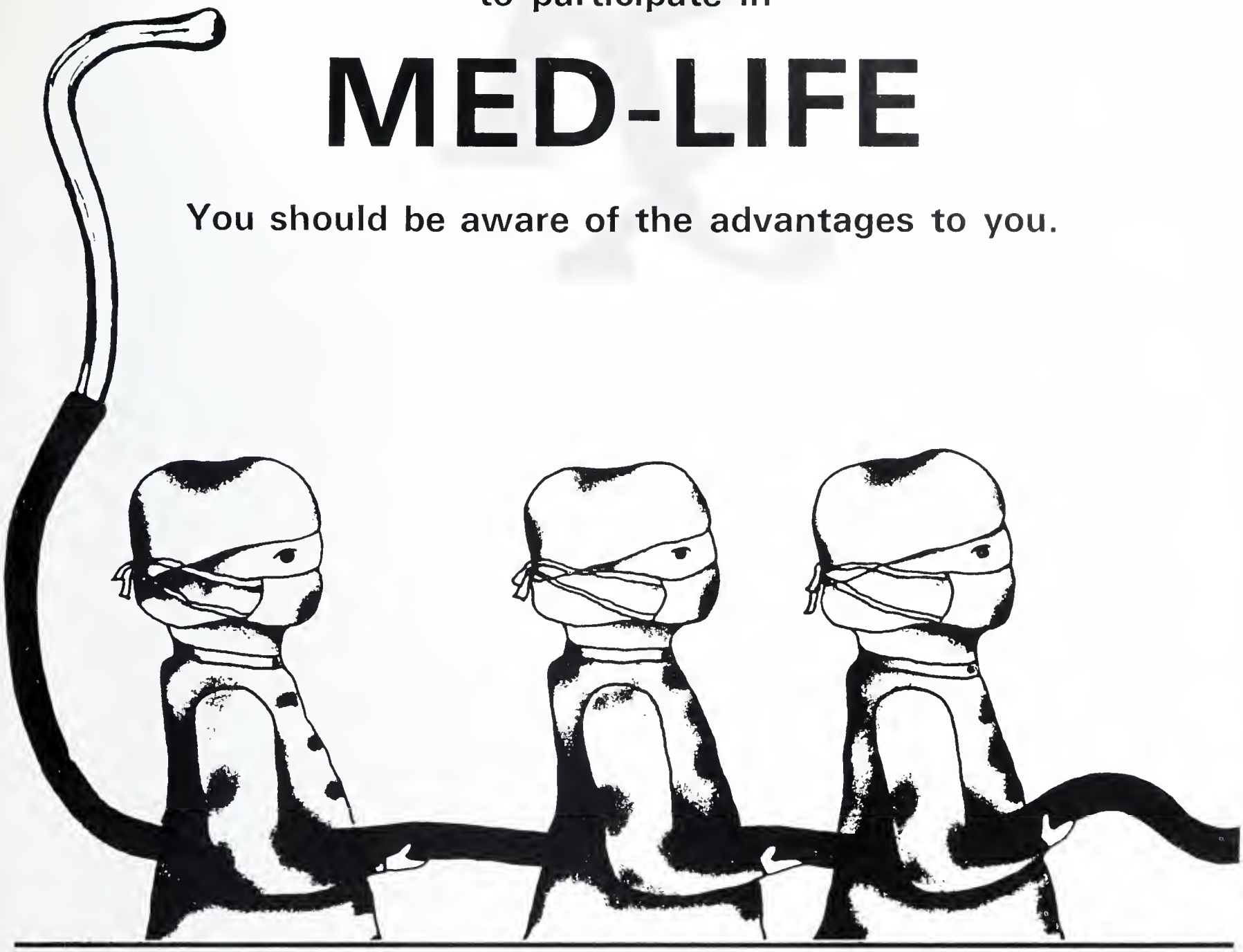

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